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VOL. 13, SEC. C. AUGUST, 1935 NUMBER 2

A COMPARISON OF THE BARGE WITH OTHER METHODS OF HARVESTING WHEAT[1]

By A. G. McCALLA[2], D. CAMERON[3] AND A. T. SINCLAIR[4]

Abstract

In 1932, in the Edmonton district, under almost ideal harvest conditions, samples harvested by the barge method were equal to or better in grade and quality than those harvested by the binder, swather or straight-combine methods. In 1933, under more adverse conditions, the barge samples were the poorest in grade and baking quality. There was some loss of material due to molding and sprouting in the barge stacks. Bin storage markedly improved the grade and quality of barge samples.

Wheat combined at 14.0% moisture content did not heat or increase in moisture content significantly during storage.

Introduction

In 1932 and 1933 a comparison of the barge method of harvesting (5) with the binder, swather and straight-combine methods was undertaken as part of an investigation on the effect of the method of harvesting on the moisture content and grade of wheat, and was extended to include the determination of milling and baking quality.

The more extensive studies on the comparison of stook-threshed, swath-combined and straight-combined samples have been reported by Larmour, Geddes and Cameron (3) and Larmour and Geddes (4). The various samples used in these studies were collected in field surveys, and hence were not of comparable origin. The results showed the highest percentage of tough and damp samples harvested by the straight-combine method and the lowest by the binder and thresher method. In 1932, the highest average grade was obtained with the binder and thresher samples and the lowest with the swath-combine samples. In 1933, these results were reversed, but the differences in the average grades were smaller than in 1932. As a consequence, the two year average gave the same relation as the 1932 results.

During the part of the harvest season over which the experiments now to be reported were carried out at Edmonton in 1932 there was but little rain, the temperature in general was high and the humidity low. Drying conditions

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were therefore close to ideal. During the same part of the season in 1933, there was considerable rain and there were periods of relatively low temperatures and high humidities. A comparison of the results obtained with the various methods of harvesting for the two years gives some idea of what might be expected in areas usually characterized by one or the other type of weather conditions.

Equipment and Material

The equipment used in harvesting the binder and barge samples was the same for the two years. The former were cut with a seven foot binder, which was also used with a 7 × 6 × 6 ft. barge in harvesting the barge samples. This type of barge equipment is somewhat unsatisfactory because more straw has to be included in the material stacked than where a header, or a binder converted to function as a header, is used, and the barge is too small to permit the most efficient stacking. Better equipment was not available, so more than usual caution was exercised in building the stacks.

The material used in both years was Marquis wheat grown on a farm located about seven miles east of Edmonton. The land was slightly rolling, but the portions of fields used were fairly uniform. In both years part of the wheat was grown as the second, and part as the first, crop after fallow. In both cases, that part of the crop on stubble land is designated Field 1 and that on fallow land Field 2.

In 1932 the fields were harvested in strips of equal width by each method. The grain in Field 1 did not ripen very uniformly, there being a small percentage of relatively green heads among the more generally ripe crop. The moisture range of grain from individual heads at the time of cutting was from 17.8 to 41.2%, the average being 25.9%. The straw was in general short and dry. The grain was combined when the moisture content of the kernels was 14.2%.

The crop on Field 2 was much heavier than that on Field 1 and development was more uniform. The moisture range of grain from individual heads was from 21.7 to 30.7%, the average being 23.6%. Harvesting was 12 days later than in Field 1. The grain was combined when the moisture content of the kernels was 13.9%.

In 1933 the first three acres was cut around Field 1 with the binder, the second three acres with the barge, the third with the swather and the remainder of the field with the combine. Harvesting by the first three methods was spread over three days. The moisture range at the time of cutting was from 15 to 53%, but the extremes represented only small spots in the field. The average moisture content was 31%. The straw was short and dry and there were but few green spots in the field. The yield of grain was about 30 bushels per acre. When the moisture content of the standing grain reached 15% combining was started, but owing to an accident with the machinery it was not completed until the next day, when the moisture content was 13.7%.

Field 2 was smaller than Field 1 and only the first three methods of harvesting were used. The grain was cut in a manner similar to that used for Field 1 except that the areas harvested with the binder and the swather were reduced to one-half acre each. The remainder was harvested with the barge. The binder and swather samples were cut when the average moisture content of the grain was 42%, but barging was not completed until the next day when the average moisture was 39%. Any advantage in maturity is therefore in favor of the barged sample. The moisture range in this field was from 24 to 51%, the straw was much heavier than, and not as dry as, that of Field 1. The yield of grain was about 35 bu. per acre.

In 1932 only the combined grain was studied after threshing. Wheat to a depth of approximately five feet was placed in a bin and temperature and moisture determinations made for a few days.

In 1933, the barge, swather and straight-combine samples from Field 1 and the barge sample from Field 2 were stored in four sections of a large bin. Individual bins, approximately 8 × 4 ft. in size, and separated from those adjoining by two-inch air spaces, were filled with grain to a depth of 4.5 ft. These lots of wheat were stored from the time of harvesting until March 1934, when they were re-sampled. The samples collected at harvest were laboratory stored, and both these, and those collected after bin storage, were used in testing for quality.

Methods

Moisture

All moisture determinations were made on approximately 80 heads taken at different places in the field of standing grain, and from different stooks, swaths or stacks of cut grain. The heads were placed in air-tight cans, taken to the laboratory, threshed out immediately, dried at 100° C. for five hours, ground and dried again at 130° C. for one hour. In sampling the barge stacks, heads were taken from three places in the stacks, a foot from the top, at the centre, and a foot from the bottom. Only heads from the inner part of the stacks were taken. Binned grain was sampled at several different places.

Temperature and Humidity

In 1932, temperature and humidity readings were taken at as near 3 p.m. as possible, and the values recorded are for this time. Maximum thermometers were left in the stacks for 1½ hr. and then read. In 1933 the temperatures recorded are the maximum for the day. The relative humidity was determined with sling psychrometers as near 10 a.m. as possible. Thermometers were placed in the barge stacks for 24 hr. before being read. The values therefore represent the maximum temperatures during the preceding 24 hr. Temperatures of binned wheat were determined by placing stick thermometers in the middle of the bins, six inches from the top, at the centre, and six inches from the bottom of the grain. The thermometers were removed only to take the readings and to lower the mercury columns.

Grades

All grading was done at the Edmonton office of the Western Grain Inspection Division and the grades are therefore official.

Protein

Total nitrogen was determined by the Kjeldahl method using mercuric oxide as catalyst. The nitrogen values were converted to protein using the factor 5.7 and corrected to the basis of 13.5% moisture.

Baking

In 1932 the simple and bromate formulas were used, while in 1933 the malt-phosphate-bromate formula (1) was added. The bromate formula included 1 mg. of potassium bromate per loaf, and the malt-phosphate-bromate included 0.3 gm. of 200 lintner malt, 0.1 gm. of ammonium dihydrogen phosphate and 1 mg. of potassium bromate per loaf.

Results

Moisture in Standing and Harvested Grain

In 1932 the moisture content of the grain in both fields was low before harvesting started. The weather was dry and warm, and all samples dried fairly rapidly (Table I). The rate of drying was most rapid in swathed grain and slowest in barge stacks, but in all cases samples were dry when threshed.

TABLE I

MOISTURE IN GRAIN, AND WEATHER CONDITIONS, 1932

Date	Moisture in grain as % of total weight				Atmospheric temperature	Relative humidity	Rainfall	Average barge stack temperature
	Standing	Swathed	Stooked	Barged				
Field 1								
Aug. 25	25.9*	25.9*	25.1*	28.4*	—	—	nil	—
27	20.4	15.8	—	24.2	68	28	nil	71
30	20.1	15.0	16.9	23.6	64	37	nil	64
31	—	—	—	—	—	—	0.01	—
Sept. 1	17.8	13.2	15.4	16.3	76	33	0.02	79
2	—	—	—	—	—	—	0.01	—
5	11.0	10.9	13.0	15.9	79	33	nil	80
7	11.0	9.6	11.5	14.2	82	—	nil	82
8	—	—	—	—	—	—	0.13	—
10	14.2	—	—	—	—	—	nil	—
12	—	11.7	12.8	13.3	75	31	nil	—
Field 2								
Sept. 5	23.6*	23.6*	24.1*	23.6*	79	33	nil	—
7	22.5	20.6	22.6	23.1	82	26	nil	83
8	16.7	12.4	—	20.2	—	—	0.13	—
10	14.5	—	15.9	17.9	82	29	nil	81
12	—	12.9	12.8	13.8	—	—	nil	—

**Average moisture content at cutting.*

In 1933 the moisture contents of grain samples were higher when harvesting started than in 1932. The weather was cooler and much damper, both because of higher humidities and more rainfall. Drying of grain was much slower except for swathed samples, and even these showed a subsequent increase as a result of a heavy rain on August 31 (Table II). The grain in the barge stacks dried more slowly than did the other lots, but was less affected

TABLE II

MOISTURE IN GRAIN, AND WEATHER CONDITIONS, 1933

Date	Moisture in grain as % of total weight				Atmospheric temperature	Average relative humidity
	Standing	Swathed	Stooked	Barged		
Field 1						
Aug. 23	31.0*	31.0*	31.0*	31.0*	—	—
26	22.1	19.3	25.6	25.3	78	54
28	16.4	16.1	19.8	28.7	89	52
30	23.9	16.4	13.9	25.8	58	74
Sept. 1	23.3	23.5	25.8	25.7	57	58
3	21.3	19.4	20.5	20.7	66	58
5	20.2	21.2	19.5	20.9	40	81
7	23.8	24.2	20.9	19.9	56	68
9	18.1	17.9	17.9	21.5	83	56
11	17.6	20.8	15.7	22.7	67	46
13	14.6	16.5	14.6	17.9	73	58
Field 2						
Aug. 23		42.0*	42.0*	—	—	—
24		—	—	39.0*	—	—
26		31.7	34.5	27.8	78	54
28		16.8	23.7	31.2	89	52
30		15.5	22.0	25.5	58	74
Sept. 1		22.3	24.1	27.8	57	58
3		30.0	24.3	29.5	66	58
5		20.8	20.0	31.8	40	81
7		23.3	21.2	27.7	56	68
9		19.3	16.9	25.5	83	56
11		20.8	15.9	22.1	67	46
13		15.4	14.6	22.3	73	58

**Average moisture content at cutting.*

by rainfall. These samples dried so slowly that two weeks after the other lots were threshed these were still tough, but it was considered inadvisable to delay threshing further. Stooked and standing grain were affected by rain, but not to the same extent as swathed material. Both dried out relatively rapidly.

Temperature of Barge Stacks

Only a few readings of the temperatures in barge stacks were made in 1932 (Table I). There was little evidence of local heating or sweating, and the temperatures were close to those of the atmosphere.

Since the grain in the stacks was higher in moisture, and the weather conditions less favorable for drying in 1933 than in 1932, a more extensive study of stack temperatures was made. Individual readings are recorded in

Table III, and it is evident that there was little if any tendency for the stacks to heat. There must have been good circulation of air throughout the stacks because the stack temperatures were similar to atmospheric temperatures

TABLE III

TEMPERATURE OF BARGE STACKS, AND WEATHER CONDITIONS, 1933

Date	Hour	Moisture	Maximum atmospheric temperature, °F.	Rainfall, in.	Average relative humidity	Temperature of stacks, °F.		
						Top	Centre	Bottom
Field 1								
Aug. 24	11.45	35.2	68	nil	58	71	71	71
25	11.20	36.7	75	nil	54	62.5	62	62
26	10.50	25.3	78	nil	54	71	71	71
27	10.35	25.8	80	nil	40	61	61	61
28	10.35	28.7	89	nil	52	61	61	61
30	16.30	25.8	58	nil	77	80	70	70
31	11.40	22.1	56	0.66	84	60	59	58
Sept. 1	11.20	25.7	57	nil	58	57	57	57
2	11.50	21.2	60	nil	57	51	50	50
3	11.50	20.7	66	nil	58	47	49	50
4	12.30	23.1	58	nil	64	57	52	49
5	11.00	20.8	40	0.07	81	50	49	48
6	11.30	20.4	43	0.09	43	40	41	43
7	11.20	19.9	56	nil	68	44	44	44
8	12.00	24.5	79	nil	62	60	60	58
9	11.20	21.5	83	nil	56	68	64	58
10	12.20	19.1	65	0.05	60	65	66	63
11	11.25	22.7	67	0.04	46	59	58	57
Field 2								
Aug. 24	11.30	38.7	68	nil	58	71	71	71
25	10.20	36.7	75	nil	54	62	62	62
26	10.30	27.8	78	nil	54	67	66	66
27	10.20	33.4	80	nil	40	62	61	61
28	10.20	31.2	89	nil	52	61	61	61
30	11.30	25.5	58	nil	74	71	70	63
31	10.20	26.8	56	0.66	84	59	58	56
Sept. 1	11.00	27.8	57	nil	58	56	57	57
2	11.05	28.2	60	nil	57	52	49	50
3	11.30	29.5	66	nil	58	49	50	50
4	12.00	25.9	58	nil	64	50	48	48
5	10.40	31.8	40	0.07	81	49	51	49
6	11.20	25.2	43	0.09	43	41	41	45
7	10.50	27.6	56	nil	68	44	44	50
8	11.30	23.4	79	nil	62	50	48	50
9	11.10	25.5	83	nil	56	65	64	63
10	12.00	23.7	65	0.05	60	60	63	63
11	11.00	22.1	67	0.04	46	59	59	58

even when there were relatively abrupt changes in the latter from day to day. The readings taken on August 30 seem higher than the atmospheric temperature, but these represent the maximum reached during the preceding 48 hours. There was a tendency to greater variations in temperature near the tops than at the centres or bottoms of the stacks. There were only a few spots where local heating occurred, these being somewhat more frequent

in the stacks in Field 2 than in those in Field 1. Most of the stacks in Field 1 were molded to some extent, and those in Field 2 were much worse. Sprouting of the grain took place in a layer two to six inches deep at the bottom of many stacks, and some of the grain from Field 2 had to be discarded. Sprouted grain was found near the top of a few stacks where rain had penetrated.

Temperature and Moisture in Stored Combined Wheat

The straight-combined wheat was relatively free from weed seeds when stored, and contained 13.9% moisture in 1932, and 14.0% in 1933. On the day when combining was done in 1932, the maximum temperature was 82° F., while in 1933 it was 77° F. The grain went into the bin at temperatures close to these figures, as indicated by the readings taken on the day, or the day after, the grain had been binned. Temperatures were taken twice daily for several days, and then daily. The results are recorded in Table IV. Moisture determinations were made regularly in 1933, but only once in 1932.

TABLE IV

TEMPERATURE AND MOISTURE OF STRAIGHT-COMBINED WHEAT IN THE BIN

Date	Hour	Maximum atmospheric temperature	Average moisture	Temperature of wheat, °F.		
				Top	Centre	Bottom
1932						
Sept. 10	21.50	82	13.9	—	80	79
11	10.50	63	—	—	83	76
11	20.00	63	—	—	83	79
12	8.00	66	14.5	—	82	83
12	21.00	66	—	—	83	74
13	8.00	78	—	—	81	79
13	21.30	78	—	—	81	71
14	14.00	63	—	—	82	75
14	19.30	63	—	—	81	77
15	21.00	62	—	—	82	74
16	21.05	62	—	—	81	72
17	22.00	68	—	—	80	74
20	11.00	51	—	—	81	70
21	18.00	58	—	—	78	65
24	8.30	61	—	—	78	66
1933						
Sept. 14	10.30	77	14.0	73	70	68
15	10.30	55	14.0	73	73	74
15	21.10	55	—	70	72	66
16	9.30	60	14.1	71	71	71
16	19.00	60	—	65	72	67
17	14.00	54	14.4	68	73	74
18	12.00	49	13.9	67	71	69
19	11.00	64	13.7	67	69	60
19	16.30	64	—	69	68	61
20	10.50	72	13.4	66	68	62
20	16.50	72	—	65	68	60
21	10.00	66	14.1	66	66	62
22	9.40	60	12.0(?)	64	66	57
23	14.45	50	14.0	62	65	52
24	16.00	41	14.0	59	58	58
25	17.00	39	14.1	57	60	53

In neither year's results is there indication of a significant increase in either temperature or moisture content of the stored grain. The temperatures decreased slowly, particularly in 1932 when the bulk of grain was much larger than in 1933. The very slow decrease in temperature is probably due to the slow diffusion of heat in bulked grain as discussed by Swanson and Fenton (6).

Grade and Weight per Bushel

The grades and weights per bushel of all samples at the time of threshing are recorded in Table V.

TABLE V

GRAIN CHARACTERISTICS

Method of harvesting	Date of threshing	Average moisture at threshing, %	Grade*			Weight per bushel, lb.
			At threshing	After laboratory storage	After bin storage	
1932						
Field 1						
Binder	Sept. 12	12.4	1°	—	—	65.0
Swather	Sept. 12	11.7	1°	—	—	60.5
Combine	Sept. 10	14.2	1°	—	—	62.0
Barge	Sept. 12	13.3	1 Hard	—	—	65.5
Field 2						
Binder	Sept. 12	12.7	1 Hard	—	—	65.0
Swather	Sept. 12	12.9	1°	—	—	64.5
Combine	Sept. 10	13.9	1°	—	—	64.0
Barge	Sept. 12	13.8	1 Hard	—	—	66.0
1933						
Field 1						
Binder	Sept. 13	14.6	1°	1°	—	64.5
Swather	Sept. 14	13.8	1°	1°	1°	63.0
Combine	Sept. 13	13.7	1°	2°	1°	64.0
Barge	Sept. 29	16.4	4 tough	3°	2°	60.5
Field 2						
Binder	Sept. 13	14.6	1°	1°	—	64.5
Swather	Sept. 14	14.0	1°	1°	—	63.0
Barge	Oct. 3	16.7	5 tough	4°	4°	61.5

**1° = No 1 Northern; 2° = No. 2 Northern, etc.*

In 1932, grades were all high, the two barge samples and one binder sample grading No. 1 Hard and the remainder No. 1 Northern. There were fairly wide variations in weight per bushel; the two barge samples were slightly heavier than the binder samples, which in turn were significantly heavier than either the swather or combine samples.

In 1933, there were marked variations in both grades and weights of the barged wheat as compared with other samples. The binder, swather and straight-combine samples all graded No. 1 Northern, while the barge samples graded Nos. 4 and 5 tough. The swather samples were somewhat bleached,

but graded No. 1 Northern. The barge samples were dark in color, musty, and contained sprouted kernels, the grain from Field 2 being worse than that from Field 1. The barge samples were also lowest in weight per bushel, while the binder samples were heaviest.

Grades were obtained again after samples had been stored in small cans in the laboratory, and the bulk of the grain bin stored. The results show that there was a marked improvement in the grade of barged grain as a result of storage. The grade of the barged grain from Field 1 was raised from No. 4 Northern to No. 3 Northern after laboratory storage and to No. 2 Northern after bin storage. The grade of the barged grain from Field 2 was raised from No. 5 to No. 4 Northern after both laboratory and bin storage. The straight-combine sample, which had graded No. 1 Northern at threshing was graded No. 2 Northern after laboratory storage, but No. 1 Northern after bin storage.

Protein, Milling and Baking Results

In 1932, the protein content of the barge and binder samples was lower than that of those harvested by the other methods (Table VI). It is possible that this can be attributed to sampling error since this relation did not hold in 1933.

The milling results are not tabulated because there was but little difference with the various samples. The swather sample from Field 1 gave slightly the highest yield, but all yields were high.

The baking results are reported in Table VI. There are no marked differences between those obtained with the simple and those with the bromate formula. In general these results parallel the protein results, the binder samples being the poorest and the swather and straight-combine samples from Field 2 the best. The protein level of these samples is in the range where a variation of 1.2% in protein must be considered important in respect to baking quality.

As already indicated, the 1933 studies were somewhat more extensive. Small samples of each lot were collected at the time of harvesting and stored in cans in the laboratory. The barge, swather and straight-combine samples which were bin stored were sampled in March, 1934. Both lots of samples were milled and baked.

The protein results (Table VI) are more uniform than those obtained with the 1932 samples. There were apparent small increases in protein content during bin storage, which may have resulted from loss of carbohydrates by respiration, or may have been the result of experimental error only. In any case, the difference between the highest and lowest protein content is only 0.6% which is not sufficient to affect the baking results markedly, since the general protein level was higher than in 1932.

The milling yields were all lower than those obtained from the 1932 samples. The barge samples gave slightly higher yields than did the others, but the differences were hardly significant.

TABLE VI

GRADE, PROTEIN AND BAKING DATA

Method of harvesting	Time of sampling	Protein (13.5% H_2O), %	Absorption, %	Simple formula		Bromate formula		Malt-phosphate-bromate formula	
				Loaf volume, cc.	Partial baking score	Loaf volume, cc.	Partial baking score	Loaf volume, cc.	Partial baking score
1932									
Field 1									
Binder	At threshing	12.4	67	598	54	621	55	—	—
Swather	At threshing	13.6	68	684	58	670	59	—	—
Combine	At threshing	13.6	67	692	58	672	53	—	—
Field 2									
Binder	At threshing	12.8	68	618	57	593	57	—	—
Swather	At threshing	14.4	69	720	58	749	57	—	—
Combine	At threshing	14.2	69	670	60	724	57	—	—
Barge	At threshing	13.1	70	650	58	665	58	—	—
1933									
Field 1									
Binder	At threshing	14.7	64	711	50	698	50	752	46
Swather	At threshing	14.7	64	648	46	576	40	608	39
Swather	After storage	15.0	64	706	48	624	51	697	43
Combine	At threshing	15.3	66	612	48	532	46	583	43
Combine	After storage	15.2	64	580	48	606	46	600	41
Barge	At threshing	14.7	66	691	42	530	36	560	35
Barge	After storage	15.0	64	670	49	640	47	694	41
Field 2									
Binder	At threshing	15.2	64	682	48	774	50	844	49
Swather	At threshing	15.1	64	731	48	618	46	638	43
Barge	At threshing	15.2	63	628	39	579	39	567	35
Barge	After storage	15.3	64	735	47	707	50	762	45

The baking results are presented in Table VI. The results obtained with the simple formula show lower loaf volumes for the straight-combine samples after both laboratory and bin storage than for other samples, and poorer scores for the barge material sampled at the time of harvesting. The low loaf volumes of combine samples persisted when these were baked by the bromate and malt-phosphate-bromate formulas, and are apparently inherently characteristic of the material. The poor scores of barge samples stored in the laboratory may be partially the result of storage in closed cans under fairly high temperatures in the laboratory. These samples when harvested smelled musty, and this smell persisted in the samples stored in the laboratory, but disappeared from those stored in the bin.

The results of the bromate baking test indicate that the binder samples were the best of those obtained at the time of threshing, but that samples

harvested by the swather and barge methods and bin stored were only slightly, if at all, inferior to the original binder samples Owing to limitation of bin space, no binder wheat was bin stored so that comparisons between the storage effects on this and other samples is not possible.

The results of the malt-phosphate-bromate test indicate that the binder samples were superior both in loaf volume and scores to the samples harvested by other methods, whether sampled at the time of threshing or after bin storage. Bin storage markedly improved the quality of barge samples, to some extent improved the swather samples, but did not improve the combine material.

Taken as a whole, these results show that the binder samples were somewhat superior to those harvested by other methods. The barge samples were tough when threshed, and made the poorest bread. The loaf volumes of straight-combine samples were consistently lower than those of binder or swather samples, and the scores consistently lower than those of binder samples. Bin storage improved the barge samples most markedly, although some of the differences noted between bin- and laboratory- stored samples may be attributed to the lack of effective aeration of the latter. Bin storage improved the swather sample somewhat, but the straight-combine sample little, if any.

Discussion

The results with the barge method obtained in the second year of this experiment are directly opposite to those obtained in the first year. The differences are due in part to differences in weather conditions and probably in part to the higher moisture content of the 1933 grain when harvested. In 1932, only 0.17 in. of rain fell during the time the barge stacks were exposed, but the grain in the stacks dried more slowly than that harvested by other methods. In 1933, from the time of cutting until September 14, when all except the barge samples were threshed, there was 0.91 in. of rain. At this time the grain in the stacks had dried insufficiently to make threshing possible, and during the two weeks which followed an additional 1.63 in. of rain fell. Thus the barge stacks were subjected to 2.54 in. of rain, and in this respect the tests were unfair to this method of harvesting. It must be emphasized, however, that since the barge samples dried more slowly than the others, it was this factor which was responsible for their exposure to extra weathering. The grain was in general free from weeds and the straw not particularly green. The higher moisture content probably had some effect on the subsequent deterioration in quality because of the longer time needed for the grain to dry.

The barge stacks were deposited on ground which was slightly damp, and the temperature and humidity conditions were in general unfavorable to drying. Some of the stacks, although built as well as the equipment would allow, leaked during the heavy rains, and the resulting wet spots undoubtedly aggravated the other conditions. The poor results with the barge material may be accounted for, then, as the total effect of damp ground, high moisture

content and poor drying conditions. The immediate cause of deterioration was not heating, but rather molding and stack-staining as described by Coleman and Rothgeb (2).

Under more generally favorable drying conditions it has been observed that barge stacks, in areas where this method is used more extensively, may be subjected to as much or more rain than were those studied in 1933, without injury to grade or quality. In such cases, however, the grain was probably drier when stacked than that in this study, and the weather conditions, other than the rain itself, were undoubtedly more favorable.

It must be concluded that under suitable conditions the barge method is satisfactory in producing wheat of high grade and quality. If conditions in a district are usually more or less like those which obtained during the 1932 studies, the method will be entirely satisfactory. If, however, conditions are in general more like those which obtained in 1933, as is the case in the Edmonton district, the method cannot be recommended.

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KERNEL TEXTURE AS AN INDICATOR OF QUALITY IN HARD RED SPRING WHEATS[1]

By O. S. Aamodt[2] and J. H. Torrie[3]

Abstract

The gray wooded soils found at Fallis, Alberta, provided a satisfactory means of obtaining a differentiation in kernel texture in hard red spring wheats. Correlation studies showed that the varieties behaved more or less similarly from year to year in kernel texture, protein content and loaf volume, but not in partial baking score. Kernel texture was indicated as being a better measure of partial baking score than protein content, while the latter was the better index of loaf volume. A close relation was found between the kernel texture of the varieties grown at Fallis and both the partial baking score and loaf volume of the same varieties grown at Edmonton. In the case of protein content determined on the Fallis material no such relation was obtained. The wheat-meal fermentation test was found to be of little value in differentiating between the baking quality of hard red spring wheat varieties.

Introduction

In the production of new wheat varieties the plant breeder has three major objectives. He desires to develop new varieties which possess desirable agronomic type, resistance to the important diseases, and high milling and baking quality. For the first two objectives, satisfactory technique has been developed for the testing of small samples in early generations but, unfortunately, such is not the case for baking quality.

At present the only course open to the plant breeder is to grow a large number of strains, until he has sufficient seed of homozygous lines for a milling and baking test. The unsatisfactory aspects of this method are the enormous amount of costly routine work that it necessitates, and the considerable amount of time required, before an indication of quality can be obtained. If such a system is not followed, and only a relatively few lines are carried on until a baking test can be made, the chances of securing new varieties excelling in milling and baking quality are greatly reduced. Thus it is clearly seen that the plant breeder is in great need of some simple method by which he may secure a fairly reliable indication of the baking quality of his various selections from the small samples obtained in the early generations of a cross. Such a method would make it possible to eliminate a large number of strains in the F_3 and F_4 generations, and to continue with only those strains that would be reasonably certain to possess satisfactory quality. In later generations, the baking test could be used for the final elimination of strains unsatisfactory in quality.

In the present paper the baking results of a number of wheats of a diverse nature, grown both at Edmonton and Fallis over a period of years, are con-

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sidered in respect to protein content and kernel texture. Inter-annual and inter-station correlations have been calculated to show the relation between the several characters for the different years, and for the different environments.

Materials and Experimental Methods

The series used in these studies was composed of a number of named varieties and hybrid strains of diverse origin. The material was grown in replicated rod-row trials at Edmonton, Alberta, on black soil; and at Fallis, Alberta, on gray soil, in the years 1931, 1932 and 1933. A brief preliminary report on the general procedure followed at the University of Alberta was made in 1933 (1).

The material was studied to find out the inherent tendencies of, and the relations among, the four characters, kernel texture, protein content, loaf volume and partial baking score*. The loaf volume and partial baking score** were determined by the bromate method of baking (10). At Edmonton in the years 1931, 1932 and 1933, 61, 29 and 28 varieties, respectively, were compared by the baking test. At Fallis for the years 1932 and 1933, 26 and 12 varieties, respectively, were tested. Baking tests were not made on the 1931 samples grown at Fallis. The method of securing the sample for the baking tests was to bulk the grain from the border rows of three-row quadruplicated rod row plots.

The protein content was calculated as total nitrogen $\times$ 5.7, corrected to a 13.5% moisture basis. The material was classified for kernel texture on a scale running from 1 to 10, 1 being completely starchy and 10 completely vitreous. Fisher (8) states that with small samples, less than 100, tests of significance based upon the standard error are often very deceptive. Consequently, the significance of the correlation coefficients reported herein was judged by the method suggested by Fisher (8). A correlation was considered significant if P, the probability of exceeding the observed value through random sampling, was less than 0.05. If the P value exceeded the 5% point, the correlation coefficient is marked with one asterisk, while if it exceeds the 1% point it is marked with two asterisks.

At Edmonton a satisfactory differentiation in kernel texture is obtained only in years characterized by a higher than average rainfall and late maturity. In order to overcome the uncertainty of obtaining a differentiation in kernel texture, it was found necessary to grow the material in an environment which would bring out inherent differences in the texture of the kernel. Observations made of the wheat coming from different parts of the province showed that samples of most wheat varieties grown on the gray wooded, or podsol soils, were very starchy in texture. Consequently in 1930 a small

*The writers wish to acknowledge gratefully the assistance of Drs. R. Newton, J. G. Malloch and A. G. McCalla in obtaining the data on laboratory quality tests.

**Partial baking score is a value used by members of the Associate Committee on Grain Research to express in a single figure an estimate of the absorption, general appearance, crumb color and crumb texture of the loaf. The perfect scores for the various characters are as follows: loaf shape, 5; crust color, 5; crumb color, 10; and crumb texture, 10. The partial baking score is calculated as: (absorption—60) + loaf shape + crust color + 2(crumb color) + 3(crumb texture).

plot of land in the gray wooded soil area at Breton, Alberta, was secured for experimental purposes. The following year, owing to the difficulty of reaching Breton, the location of the experimental plots was changed to Fallis, Alberta, which is located in the gray wooded soil area fifty miles west of Edmonton. The nature and composition of the soils of this area are described by Wyatt Newton and Mather (24). The results of preliminary experiments indicated that the kernel texture of wheat grown at Fallis provides a good differential reaction for baking quality. In order to test more thoroughly the above observations, the experiments and calculations reported in this paper were made.

Literature Review

Shollenberger and Coleman, 1926 (21) report that the vitreous kernels of a wheat sample are superior in loaf volume to the starchy kernels. Coleman, Dixon and Fellows, 1927 (6) and Mangels, 1927 (14) conclude that kernel texture is of questionable value as an indication of baking strength. Hayes, Immer and Bailey, 1929 (11) found that kernel texture was neither significantly nor consistently related to loaf volume.

Significant positive correlations between protein content and loaf volume for either, or both, spring and winter wheats, have been reported by a number of investigators (4, 5, 6, 12, 13, 25, 26). Larmour, 1930 (12) concludes that the relation between these two characters in sound wheat is close enough to justify the use of the protein test as a factor in the classification of hard red spring wheats. Hayes, Immer and Bailey, 1929 (11) found, from a study of a number of wheats of a diverse nature, no significant relation between protein content and loaf volume.

A review of the literature on the relation between kernel texture and protein content shows a considerable difference of opinion. Several investigators (9, 20–23) obtained a significant relation between vitreous kernel texture and high protein content Other investigators (6, 14, 15, 18) report that kernel texture is of questionable value as an index of protein content. Newton, Cook and Malloch, (16) found some evidence of a curvilinear relation existing between vitreousness of kernel and protein content.

Experimental Results

In the varietal studies the four characters, grain texture, protein content, loaf volume and partial baking score were compared. Correlation studies were made in order to measure both the tendency of the varieties to react similarly, when grown during the different years and at the different stations, and the relation between the several characters. The significance of the correlation for any character from year to year gives an indication of its heritable nature and also an expression of its reliability as an index of quality. The degree of association among the several characters was studied primarily to find out if the relations between kernel texture, protein content, loaf volume, and partial baking score were sufficiently high to warrant their use as indices of baking quality.

TABLE I

INTER-ANNUAL CORRELATIONS

Variables correlated	Edmonton					
	1931–32		1931–33		1932–33	
	No. of samples	r	No. of samples	r	No. of samples	r
Protein content	35	+0.440**	22	+0.612**	28	+0.252
Kernel texture	—	—	—	—	—	—
Loaf volume	18	+0.519*	17	+0.725**	18	+0.687**
Partial baking score	18	+0.373	17	+0.213	18	+0.101

Variables correlated	Fallis					
	1931–32		1931–33		1932–33	
	No. of samples	r	No. of samples	r	No. of samples	r
Protein content	28	+0.422*	11	+0.699*	11	+0.559
Kernel texture	24	+0.360	11	+0.607*	11	+0.817**
Loaf volume	—	—	—	—	11	+0.537
Partial baking score	—	—	—	—	11	+0.280

*P value exceeds 5% point. **P value exceeds 1% point.

The data in Table I and Table H give the inter-annual and inter-station correlations for the four characters studied. The correlations of protein content for the comparisons made were significant between the years 1931 and 1932, and 1931 and 1933, both at Edmonton and Fallis; and between Edmonton and Fallis for the years 1931 and 1932. For kernel texture the correlations, for the comparisons studied, were significant between the years 1931 and 1933, and 1932 and 1933 at Fallis, and between Edmonton and Fallis for 1931. The other correlations, both of protein content and kernel texture, although not statistically significant, indicate the trend of relationship. The correlations for loaf volume between any two years were significant, except between 1932 and 1933 at Fallis. A non-significant negative

TABLE II

INTER-STATION CORRELATIONS (EDMONTON AND FALLIS)

Variables correlated	1931		1932		1933	
	No. of samples	r	No. of samples	r	No. of samples	r
Protein content	52	+0.562**	28	+0.554**	11	+0.402
Kernel texture	89	+0.382**	—	—	—	—
Loaf volume	—	—	17	−0.007	11	+0.749**
Partial baking score	—	—	17	+0.129	11	+0.719*

*P value exceeds 5% point. **P value exceeds 1% point.

correlation was obtained for loaf volume in 1932, between Edmonton and Fallis. With partial baking score the only significant correlation obtained was that between Edmonton and Fallis for 1933. These correlations indicate that, for the characters studied, with the exception of partial baking score, the varieties used behave more or less similarly for the different years at Edmonton and Fallis. The lack of correlation between the partial baking scores for the different years at Fallis and Edmonton indicates that some of the components of this character fluctuate rather widely from season to season. This indicates that for the conditions of the experiment, partial baking score in itself is not a good measure of quality. However, when it is used in conjunction with loaf volume it is of considerable value.

All possible inter-relations between the four characters studied, at both Edmonton and Fallis for the years 1931 to 1933, were determined by means of both the simple and second order partial correlation coefficients. The results are given in Table III. Partial correlations were not calculated for the years 1932 and 1933 at Edmonton, because several of the variables did not appear to be related to any of the other variables, as determined by means of the simple product-moment correlation coefficient.

TABLE III

CORRELATIONS BETWEEN SEVERAL INDICES OF BAKING QUALITY

Variables correlated†	Simple correlations				Variables correlated	Partial correlations			
	Edmonton		Fallis			Edmonton		Fallis	
	No. of samples	r	No. of samples	r		No. of samples	r	No. of samples	r
1931									
TP	61	+0.410**	88	+0.447**	TP.VS	61	+0.340**	—	—
TV	61	+0.439**	—	—	TV.PS	61	−0.124	—	—
TS	61	+0.666**	—	—	TS.VP	61	+0.586**	—	—
PV	61	+0.439**	—	—	PV.TS	61	+0.379**	—	—
PS	61	+0.270*	—	—	PS.TV	61	−0.220	—	—
VS	61	+0.654**	—	—	VS.TS	61	+0.571**	—	—
1932									
TP	—	—	26	+0.588**	TP.VS	—	—	26	+0.278
TV	—	—	26	+0.408*	TV.PS	—	—	26	−0.095
TS	—	—	26	+0.702**	TS.VP	—	—	26	+0.541**
PV	29	+0.405*	26	+0.737**	PV.TS	—	—	26	+0.637**
PS	29	+0.060	26	+0.608**	PS.TV	—	—	26	+0.194
VS	29	+0.076	26	+0.481*	VS.TS	—	—	26	+0.104
1933									
TP	—	—	12	+0.675*	TP.VS	—	—	12	+0.566
TV	—	—	12	+0.482	TV.PS	—	—	12	+0.079
TS	—	—	12	+0.586*	TS.VP	—	—	12	+0.327
PV	28	+0.032	12	+0.391	PV.TS	—	—	12	+0.079
PS	28	+0.233	12	+0.435	PS.TV	—	—	12	+0.014
VS	28	+0.797**	12	+0.668*	VS.TS	—	—	12	+0.541

*P value exceeds 5% point. **P value exceeds 1% point.

†T = kernel texture; P = protein content; V = loaf volume; S = partial baking score.

The simple correlation coefficients between kernel texture with protein content, loaf volume, and partial baking score were significant in all cases, with the exception of that with loaf volume, at Fallis, in 1933. Holding loaf volume and partial baking score constant, the correlations between kernel texture and protein content are lowered in every instance, and only at Edmonton for 1931 is the correlation statistically significant. In all comparisons when protein content and partial baking score are held constant, the correlations between kernel texture and loaf volume are not significant. Partial correlations of kernel texture with partial baking score, holding protein content and loaf volume constant, are significant in two of the three comparisons made. Similarly the partial correlations of protein content with loaf volume, holding the other variables constant, are significant in two of the comparisons. Between loaf volume and partial baking score, the partial correlation is significant only at Edmonton for the year 1931. The results indicate that kernel texture gives a better indication of partial baking score than does protein content, while protein content is the better index for loaf volume.

At Edmonton in most seasons it is impossible to obtain a satisfactory differentiation in kernel texture, while at Fallis large differences in the texture of kernel between varieties are secured every season. Consequently it is of first importance to know the relation existing between Fallis kernel texture and Edmonton baking quality. The data in Table IV give the correlation

TABLE IV

INTER-STATION CORRELATIONS (EDMONTON AND FALLIS)

Edmonton	Fallis			
	Kernel texture		Protein content	
	No. of samples	r	No. of samples	r
1931 loaf volume	49	+0.323*	51	+0.196
1932 loaf volume	16	+0.699**	14	+0.284
1933 loaf volume	11	+0.920**	11	+0.498
1931 partial baking score	49	+0.301*	51	+0.233
1932 partial baking score	16	−0.184	14	−0.280
1933 partial baking score	11	+0.758**	11	+0.211

*P value exceeds 5% point. **P value exceeds 1% point.

coefficients of kernel texture and protein content for a number of spring wheat varieties grown at Fallis, with the loaf volume and partial baking score of the same varieties grown at Edmonton. Protein content at Fallis is shown to have no significant relation with loaf volume and partial baking score at Edmonton. For the three years of the test Fallis kernel texture, however, was strongly correlated with loaf volume at Edmonton. The correlation coefficients of Fallis kernel texture and Edmonton partial baking score were significant for 1931 and 1933, but not for 1932.

From the data presented it may be concluded that the kernel texture determined on wheat varieties grown at Fallis is a reliable index as a general measure of the baking quality determined on the same varieties grown at Edmonton. As such it is considered to be of great value in obtaining a preliminary indication of the baking quality of hybrids in the early generations. In plant breeding studies conducted at the University of Alberta, kernel texture as determined on wheat grown at Fallis is being used to a considerable extent as a preliminary method of eliminating strains of undesirable baking quality.

The method employed is to plant at Fallis a replicate of all hybrids in single five-foot rows. At harvest all the plants in a row are bulked, threshed and classified for kernel texture, as described previously. The lines or strains possessing a low texture index are discarded, while those with a high texture index and of desirable agronomic type are retained for further tests. This method is continued for several years and by the fifth or sixth generation milling and baking tests are run as a final test for quality. The great advantage of this method is that it enables the elimination in the early generations of a large number of strains of poor baking quality.

The wheat-meal fermentation time test developed by Saunders and Humphries (19), Pelshenke (17) and Cutler and Worzella (7) was tried in order that this method might be compared with baking tests and the kernel-texture test suggested in this paper. The usual procedure was followed, but much trouble was encountered in determining the "fermentation time" or end point. There was little difficulty in differentiating soft, low quality wheats from hard or bread wheats. In the latter group the results with the wheat-meal fermentation method were so variable that it appeared to be of little value to the plant breeder working with hard red spring wheats. With these wheats small pieces of dough would break off, or hang down in strings, long before there was any general deterioration of the dough ball. The difficulty in securing uniformity in molding of the dough ball was an important factor which could not be overcome and which appeared to be the cause of this irregular and variable breaking down of the dough.

There were marked differences in the baking quality of the wheat varieties grown at both Edmonton and Fallis. These varietal differences were readily demonstrated by the baking and kernel-texture tests, but not by the wheat-meal fermentation test for either the soft or hard wheat groups. As a result of these experiments it was concluded that the wheat-meal fermentation test is of little value to the plant breeder or cereal chemist, in differentiating between the quality of varieties of hard spring wheats or the quality of the same variety when the grain is produced under such extreme environmental conditions as those existing at Edmonton on the black soil, and at Fallis on the gray soil.

Discussion

The results of the present paper indicate that, for hard red spring wheat varieties grown under environmental conditions such as the gray soils, which bring out consistently the inherent differences in kernel texture, this character

is of considerable value as an index of baking strength. Correlation studies with diverse wheat varieties show that kernel texture is inherited from year to year to the same extent as loaf volume and protein content. Larmour (12) from studies of protein content and loaf volume determined by both the simple and bromate methods, concludes that the relation between protein content and loaf volume (bromate) is sufficiently high to warrant the use of protein in evaluating the quality of hard red spring wheats. The data reported herein also lend support to the evidence that protein content is of value as an indication of baking quality as measured by the bromate bake.

Since both kernel texture, as determined on samples grown at Fallis, and protein content are correlated with baking quality, the use of both of these methods would give a better measurement of baking quality than either alone. Protein content can be determined accurately from small samples of grain. However, the cost prohibits its use in estimating the quality with hybrid populations, where a large number of individuals are grown. In order to secure an estimate of baking quality from kernel texture, all that is necessary is to grow the material under a differential environment and classify for texture after threshing. The data obtained from the varietal studies reported earlier in this paper indicate that kernel texture is a better indication of baking quality than is protein content. For these reasons the kernel texture of wheat grown at Fallis is a more practical index of baking quality than is protein content for hybrid populations.

Aamodt and Torrie (2), and Aamodt, Torrie and Wilson (3), by genetic studies of several series of crosses show clearly that the kernel texture of wheat grown at Fallis is a heritable character. The uncertainty of securing a differential reaction at Edmonton for this character demonstrates the necessity of growing the material in a differential environment in order to obtain data of value. The small variations in kernel texture which commonly occur in certain years in the black and brown soil areas of western Canada are of little value for the purpose of estimating baking quality. However, as indicated from this study, the gray wooded soil area of Alberta affords an excellent environment for obtaining a differential reaction for kernel texture every year.

In certain years early frosts do considerable damage to the kernel before it has had an opportunity to mature properly. This is particularly true of hybrid material grown in spaced nursery rows in the earlier segregating generations. When wheat is severely injured by frost, the ordinary baking tests are of little value in differentiating between varieties. Under these conditions the differences in texture, when the grain is grown on gray soil, are still observable and can be used to estimate differences in quality when all other methods fail.

The method of obtaining an estimate of baking quality by securing a differentiation in kernel texture is not put forward as a general substitute for the milling and baking test. However, the authors do believe that the kernel-

texture index determined on samples grown at Fallis, on the gray wooded soil, is of considerable value in obtaining a preliminary indication of baking quality in the early generations of hybrid material.

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A COMPARATIVE COLOR TEST FOR COUMARIN AND MELILOTIC ACID IN *MELILOTUS* SPECIES[1]

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Abstract

The coupling reaction between phenolic bodies and diazotized *p*-nitraniline in alkaline solution is shown to be of value in the detection and estimation of the coumarin and melilotic acid content of small quantities of sweet clover. The intensity of crimson color developed on treatment of pure coumarin solutions is shown to be proportional to the concentration of coumarin and to be adaptable to colorimetric measurements. For practical purposes it is shown that this reaction can be used for estimation of coumarin and melilotic acid together.

It is possible to obtain the crimson color directly with plant extracts containing coumarin or melilotic acid, and a tentative method enabling comparisons to be made among small amounts of plant material is outlined. A comparison between results obtained by the colorimetric method outlined and by that of Obermayer shows that the latter method is open to several serious errors in the estimation of coumarin.

Introduction

The sweet clovers (*Melilotus alba* and *M. officinalis*), used as leguminous forage crops, are characterized by the presence of a "bitter principle" which affects their palatability as stock feed, and which imparts to the plant a distinct, but not unpleasant, vanilla-like odor. This property has long been associated with the presence of coumarin or its derivatives in the tissue of the plant. Individual plants appear to differ in their content of coumarin as well as in other characteristics, and attempts have been made to improve the plant by selection, particularly to secure a non-bitter variety. In 1913, Obermayer (7) stressed the need for a chemical test for coumarin in sweet clover which could be used in the selection of strains for breeding work. He submitted a procedure for the quantitative determination of coumarin which has been generally accepted as the standard method for use with plant material. This method consists in the main of distillation of the coumarin from an ether extract of the plant material and titration with standard potassium permanganate solution. It is difficult to obtain satisfactory replication of results by Obermayer's procedure and consequently a number of other methods have been proposed. Recently Duncan and Dustman (3) have shown that ether extraction of the plant is unsatisfactory, and they have proposed a modification of Obermayer's method in which the ether extraction of the original plant material is replaced by direct distillation under reduced pressure.

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Another method recently published is that of Kanewskaja and Fedrowa (4) whose technique is based on the separation of coumarin and melilotic acid from an ether extract of the material by converting them to their sodium salts, which are not soluble in ether. The coumarin and melilotic acid are separately recovered by acidification and subsequent ether extraction at different degrees of acidity. They are then weighed. This method is open to the criticism applied by Duncan and Dustman (3) to Obermayer's method, with regard to the unsatisfactory nature of the initial extraction with ether. In following out this method here, difficulty was found in obtaining a separation of the coumarin and melilotic acid from the plant pigments.

All these methods are slow and require large amounts of material and considerable equipment. Consequently none of them are suited to the rapid testing of single plant selections. To be of practical importance in plant-breeding work, a test for coumarin in sweet clover should be: (i) applicable to small amounts of material, so that individual plants may be tested without injury, or a small portion of the seed yield from a single plant may be analyzed; (ii) simple and inexpensive and rapid enough to permit treatment of a large number of samples daily; and (iii) capable of relatively quantitative interpretation. In this paper is presented a relatively quantitative colorimetric test for coumarin in sweet clover. It is hoped that the test will be of use to the plant breeder.

Occurrence and Combination of Coumarin Compounds in Plant Tissue

The forms in which coumarin exists in plant tissues have been the subject of much investigation. In the tonka bean, coumarin is often found in pure crystalline form between the seed cotyledons, and extraction is carried out on a commercial scale. In *Melilotus officinalis*, coumarin seems to be found usually combined with or associated with melilotic acid. Zwenger and Bodenbender (9) state that coumarin in *Melilotus* occurs in combination with melilotic acid. Later, Wischo (8) described a distillation of dried *Melilotus* from which were obtained coumarin, melilotic acid and its anhydride, and a little melilotaldehyde. In later years evidence has been given to show that these compounds occur in part in the form of glucosides. Bourquelot and Hérissey (1) obtained from *Melilotus* and *Asperula* glucosides which on hydrolysis with emulsin yielded coumarin. They obtained a larger percentage extraction of coumarin from an acid hydrolysis than from a boiling aqueous extract. Navez (6) isolated from *Melilotus albus* and *M. altissimus* a substance which he called coumarigenin, and which appeared to be a glucoside yielding coumarin hydrocoumarate and *d*-glucose on hydrolysis. Thus, coumarin is to be found in plant tissue with other associated compounds of the general type of hydroxyphenylpropionic or hydroxycinnamic acid.

Melilotic acid, which can be prepared readily by reduction of coumarin, has a peculiar honey-like odor, typical of sweet clover, and, although sharply acid in taste, is not as bitter as pure coumarin. By distillation of melilotic acid is obtained an oily substance which is quite bitter and similar in taste

to coumarin. On hydrolysis this substance is converted to melilotic acid. It is open to question whether the bitter taste of sweet clover is attributable to coumarin alone or whether it is caused by both coumarin and melilotic acid. Though the exact relation between these substances in the plant is not known, it is evident that they usually occur together, and therefore a chemical test that would indicate the presence of both these substances might be just as useful from the plant breeder's standpoint as a determination of coumarin alone.

The Azo Coumaric Acid Color Reaction

It seemed evident from the foregoing considerations that a delicate color reaction, capable of application to small amounts of plant extract, would be highly desirable, and to this end the possibilities of the coupling reaction with coumarin giving azo dyes or colors was investigated. Chakravarti (2) in 1931 reported the preparation of dyes by the coupling of diazotized arylamines with coumarin in sodium hydroxide solution, and stated that the majority of dyes so formed are yellow or red. Accordingly, solutions of various arylamines were diazotized and treated with coumarin in alkaline solution and the colors observed. It was decided to use *p*-nitraniline in this work because the color reaction appeared to be very sensitive. A distinct crimson color was obtained with concentrations of pure coumarin as low as 1 : 125,000, and the color is detectable in concentrations of less than 1 : 1,000,000.

The crimson color is developed only after the addition of alkali and evidently depends on the breaking of the lactone structure of the coumarin to give a free phenolic hydroxyl group. After the color has been developed, addition of acid turns the solution yellow, and if the solution is concentrated enough, gives a precipitate of an orange colored compound. The crimson color is not removed from an alkaline solution by ether, but when the solution is acidified the compound dissolves in the ether layer. The point of change from red to yellow is quite sharp, and the substance may be said to have indicator properties. Prolonged boiling of the solution does not destroy, but intensifies the color. On cooling the solution, the original intensity of the color is restored.

The Applicability of the Reaction for Coumarin Detection in *Melilotus* Species

Owing to the extreme sensitivity of the test, it was thought that it would prove an excellent one for identification of coumarin in sweet clover if similar results could be obtained with a plant extract. A number of extracts of sweet clover, aqueous, alcoholic and ethereal were treated with the reagents and all developed a crimson color which was not appreciably masked by the green color of the extract. It was also found that an extract made from a single leaf of the plant gave a distinct crimson color, and that a direct staining test could be obtained on a cross section of a stem of sweet clover. The question at once arose "Is this red color due to coumarin only or to other compounds likely to be present in the plant extract?"

Melilotic acid is very frequently associated with coumarin and on account of the close similarity in the structure of these two substances, they should behave similarly in respect to this reaction. Furthermore, any other phenolic or arylamine derivatives, if present, might be expected to couple and produce color. However, the number of such substances likely to be found in the extracts of sweet clover is limited and, as will be shown later, it is improbable that any except melilotic acid, coumarin and closely related derivatives are to be found in appreciable quantity in this plant.

Experimental

Specificity of Test

A number of aryl compounds were treated with the coupling reagents and any showing a red color were tested further in a more dilute solution (1 : 125,000). Of the substances tested, saligenin, guaicol, phenol, coumarin and melilotic acid gave distinct red colors at this concentration, but tyrosine gave only a faint red. However, if phenol, tyrosine or any other interfering substance is present in the sweet clover extracts, one would expect them to be present in some or all of the other grasses and legumes similar to sweet clover. Thus, if a number of other plant materials are extracted and tested with the diazotized solution and a red color is not produced, it would be reasonable to conclude that a red color produced in sweet clover is due to coumarin or melilotic acid and not to any other compound. Results given in Table I substantiate this conclusion.

TABLE I

COLORS OBTAINED BY TREATING ALCOHOLIC EXTRACTS OF VARIOUS PLANTS WITH STANDARD DIAZONIUM SOLUTION IN ALKALI

Alcoholic extracts tested	Color obtained
Brome grass	Pale yellow to green
Trigonella	Pale yellow to green
Oats	Pale yellow to brown
Western rye	Pale yellow to brown
Reed canary grass	Pale yellow to brown
Sweet grass	Pale yellow to brown
Couch grass	Pale yellow to brown
Barley	Pale brown to green
Wheat	Pale brown to green
Alfalfa no. 1	Pale brownish yellow to olive
Alfalfa no. 2	Pale brownish yellow to olive
Crested wheat grass	Pale light brown
Alpha sweet clover	Brilliant crimson red
Redfield yellow sweet clover	Deep crimson red
Arctic sweet clover	Deep crimson red
Albatrea sweet clover	Deep crimson red
Alfalfa with 0.0002 gm. of coumarin added	Deep crimson red

It can be seen from Table I that the diazonium solution gave no indication of crimson coloration except in the case of the *Melilotus* species, and in the extract to which pure coumarin had been added. With these exceptions, the extracts tested gave pale colorations ranging from olive green to reddish brown. Undoubtedly this shows that coupling by some unknown compounds is taking place in all these extracts, but that these can be present in only small amounts. The addition of small amounts of coumarin to any one of these extracts is sufficient to give a distinctive red color to the solution when treated. Thus, it is comparatively safe to assume that

when the test is applied to extracts of *Melilotus* species, the development of a distinctive red or crimson color is indicative of the presence of coumarin and melilotic acid or their simple derivatives. A number of extracts of seeds of various grasses gave similar results. Only in those of *Melilotus* species was there any indication of a crimson color.

Standardization of the Test

It was decided to investigate the possibilities of developing a quantitative colorimetric test for coumarin and melilotic acid in sweet clover. A number of colorimetric determinations of organic compounds by the use of diazonium salts have been devised in late years. Koessler and Hanke (5) in the course of their study on reactions of *p*-diazobenzene sulphonic acid standardized a method of diazotizing suitable for such work. The technique outlined in this paper for diazotizing is largely based on their method, with minor modifications.

In order to test small quantities of material, it was necessary to have conditions suitable for obtaining a maximum color with 0.001 gm. of coumarin or less. Since the reaction is a coupling one, the proportion of the diazonium compound used must be sufficient to react with the maximum amount of coumarin likely to be present in the extract. Secondly, as toward the neutral point the color changes from red in base to yellow in acid, it is necessary to have an excess of alkali for each test. Too great an excess of alkali, however, causes a decrease in the intensity of the color. For all colorimetric comparisons the final volumes of the test solutions must of necessity be equal. The final volume can be varied to suit the quantity of material tested. For colorimetric comparison the solution should be relatively dilute, for the colors produced are too intense in concentrated solution for accurate matching.

The procedure was as follows. To not more than 40 cc. of the solution to be tested for coumarin, 2.5 cc. of 1.1% sodium carbonate solution was added and the liquid was heated nearly to boiling on a water bath, in order to permit equilibrium between the lactone and acid configuration of the coumarin molecule to be speedily reached, and allowed to cool slowly. To this, 5 cc. of the diazonium solution* was added and the volume made up to 50 cc. with distilled water.

The solutions were allowed to stand for 30 min. at room temperature before comparing the colors to permit equilibrium to be established.

**Diazonium solution*

Solution A—*p-Nitraniline hydrochloride.*

p-Nitraniline (3.5 gm.) was dissolved in 45 cc. of 37% hydrochloric acid and the solution was diluted to 500 cc. with distilled water, and filtered. The solution will keep indefinitely if stoppered.

Solution B—*Sodium nitrite*

Sodium nitrite (5 gm.) was dissolved in 100 cc. of distilled water. This solution is not as stable as Solution A and should be renewed frequently. It is best to keep the solution away from the light and in a dark bottle.

The diazonium solution, which the authors have found convenient to make up in 100 cc. lots, was prepared as follows: Stock solutions A and B were placed on ice before preparation of the diazonium solution. Solution A (3 cc.) and Solution B (3 cc.) were placed in a 100 cc. volumetric flask which was immersed in an ice bath for five minutes. To this, 12 cc. of Solution B was added, the flask shaken and returned to the ice bath for an additional five minutes. The flask was then filled to the 100 cc. mark with ice-cold, distilled water and kept on ice for 15 min. before using. If kept on ice, the diazonium solution will remain stable for at least 24 hr.

Quantitative Nature of the Color Test

In a number of preliminary experiments with solutions of pure coumarin it was observed that the intensity of the crimson color appeared to be proportional to the concentration of coumarin, and after a standard procedure had been developed for the preparation of the diazonium solution, a series of quantitative comparisons was made on coumarin and on melilotic acid, and these were compared with results obtained with Obermayer's test.

To ascertain whether the colors developed with coumarin were proportional to the concentrations a series of solutions of known coumarin content was made up and treated in the prescribed manner. The colors developed were matched against one another in a standard colorimeter. The results given in Table II show that, over a wide range of concentrations, under the conditions of the test the intensity of color is proportional to the concentration.

TABLE II

Colorimetric measurements of coumarin concentration

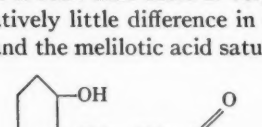
Initial concentration of coumarin, mg./38.5 cc.	0.20	0.30	0.40	0.50	0.60*	0.70	0.80	0.90	1.0
Calcd. concn. from colorimetric measurements, mg./38.5 cc.	0.20	0.30	0.42	0.53	0.60*	0.71	0.80	0.89	0.99

Note:—*The coumarin solution was prepared so that 1 cc. of solution was equivalent to 0.2 mg. of coumarin. (The final solutions in each case were diluted to 38.5 cc. instead of 50 cc. as presently recommended.)*

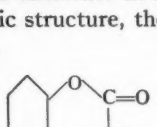
**Used as standard.*

These and other similar results show the possibility of determining coumarin quantitatively by the colorimetric method. However, it can be seen that in testing sweet clover, a difficulty arises owing to the likelihood that melilotic acid is present as well as coumarin. If this were so, the intensity of the crimson color would be proportional to both. However, in considering the test from the standpoint of the plant breeder, a combined determination would present no handicap, since evidently these substances occur together and are the cause of the bitter taste which is so undesirable.

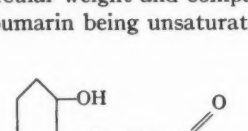
From an inspection of the formulas of melilotic acid (I, mol. wt. 166.08), coumarin (II, mol. wt. 146.05) and coumaric acid (III, mol. wt. 164.06), it is seen that there is very little difference in molecular weight and comparatively little difference in organic structure, the coumarin being unsaturated and the melilotic acid saturated.



(I)



(II)



(III)

As the development of the crimson color with the diazonium solution is primarily due to the coupling with the hydroxyphenyl grouping, it would be

expected that the color developed by a given quantity of coumarin would be about the same as that developed by a similar concentration of melilotic acid, and that for practical purposes these substances could be determined together. This was shown to be so in the following experiment.

A solution of coumarin and a solution of melilotic acid were made up so that 1 cc. of each was equivalent to 0.0002 gm. of reagent. A number of test tubes were set up containing definite amounts of coumarin and melilotic acid, and also some with mixtures of the two. These were treated and compared, one against another, and within the limit of error of the readings of the colorimeter these appeared to coincide with a mean ratio of coumarin : melilotic acid : : 1 : 1.012. On the basis of the molecular weights of coumarin and melilotic acid the ratio should be 1 : 1.137 and on the basis of coumaric acid to melilotic acid 1 : 1.0123. It can be seen that whereas coumarin does not develop the crimson color to the same extent as melilotic acid on a molecule-to-molecule basis, yet for practical purposes the results obtained by comparing a mixture of coumarin and melilotic acid with a standard coumarin solution can be interpreted as the concentration of the coumarin–melilotic-acid mixture. Further work in separating the coumarin from the melilotic acid might profitably be done. The work of Kanewskaja and Fedrowa (4) in this respect should be followed up.

Comparison of Obermayer and Colorimetric Tests

Pure Coumarin

As the method of Obermayer has been accepted as a standard procedure for coumarin determination, a comparison of the two methods was made. This was first done in the case of pure coumarin solutions.

Pure coumarin (0.1 gm.) was distilled from a calcium chloride solution, as in the Obermayer procedure. Aliquot portions were tested for coumarin by Obermayer's titration and also by colorimetric comparison with standard coumarin solutions. The results are shown in Table III.

TABLE III

Comparision of colorimetric and volumetric determinations of coumarin

Weight of coumarin originally used, gm.	0.1000
Weight of coumarin found by Obermayer titration on distillate, gm.	0.0973
Weight of coumarin found by colorimetric comparisons on distillate, gm.	0.0978

These results show that the intensity of the crimson color produced is virtually proportional to the concentration of coumarin present in the solution, and that the color test is quantitative.

Coumarin in Sweet Clover

The method was next applied to the determination of the coumarin in sweet clover, and a comparison was made with results obtained by Obermayer's method. By using the Obermayer distillation procedure it is possible

to obtain from an ether extract a colorless distillate in which the crimson color can be developed nicely. However, this would entail as much time and equipment as the Obermayer method itself, and would be open to the same criticism. On the other hand, when attempts are made to produce the color directly in an alcoholic or aqueous plant extract, solutions of various plant pigments to some extent mask the true crimson color of the azo dye. However, by using the yellow-green extract of a non coumarin-bearing plant and by adding to it known quantities of coumarin, it is possible to set up a series of colors whereby a comparison of sweet clover extracts can be made. A tentative procedure adopted for this estimation was as follows: 1 gm. samples of air-dried, ground leaves and stems of sweet clover, and either alfalfa or some other non coumarin-bearing plant were heated just to the boiling point with 25 cc. of 95% alcohol. The solutions were shaken for half an hour, made up to 50 cc. with water and shaken for another half hour. The plant material was allowed to settle for one hour. Then 5 cc. of each solution was treated with the color reagents in the prescribed manner and made up to 50 cc. with water. To the alfalfa extracts there was added enough standard coumarin solution to set up a series of standards containing from 0.0001 to 0.001 gm. of coumarin. The colors developed in the sweet clover were matched against the alfalfa standards and the results expressed as percentages of coumarin. As stated before, this would include melilotic acid if present.

Duncan and Dustman (3) have shown that the ether extraction method for obtaining coumarin from sweet clover is open to error, owing to the incomplete extraction of the coumarin, and they advocate in place of this method steam distillation of the plant material at 140 mm. pressure, to be followed by the Obermayer permanganate titration. This method, though not as long as the Obermayer ether extraction procedure, is technically difficult, and is open to the same sources of error as the permanganate titration, namely, that any other substance which distils over and which is oxidizable by potassium permanganate will affect the titration. Obermayer recognized this difficulty, but he showed that the amount of impurity in the distillate from the ether extraction was small and could be neglected. According to Obermayer the ether extraction method is less likely to introduce impurities in the distillate than is direct steam distillation of the plant material. Experiment showed, however, that a considerable amount of insoluble waxy material distils over from the ether residue, and he recommended filtering it off. However, with the direct steam distillation of the plant material, Duncan and Dustman advocate removing the impurities from the distillate with lead acetate, but they state that plant materials containing only small amounts of coumarin and considerable amounts of volatile reducing substances not precipitated by lead acetate cannot be determined accurately by this method.

Another peculiarity in Obermayer's method and one which he mentions himself is brought out in a consideration of the melilotic acid in the sweet clover. This presumably would be extracted by the ether and when the ether

residue was distilled should appear in the distillate as melilotic anhydride, which has a melting point of 25° C. Obermayer states that the crystalline material recovered by an ether extraction of the distillate he obtained had a melting point of 67–67.5° C., and he concludes that there could have been no other compound of coumaric acid except coumarin in the material he investigated. This appears at variance with the results of other workers who have shown that melilotic acid is present in sweet clover.

In order to investigate this point and to ascertain whether melilotic acid would be carried over in the steam distillate and oxidized by permanganate the following experiment was performed: 0.1 gm. of melilotic acid was weighed in a distillation flask and the regular Obermayer distillation was carried out. After about half an hour of distillation considerable foaming occurred and the contents of the flask became yellow to golden yellow. It was impossible to distil over as much liquid as when distilling coumarin, but when about 50 cc. was left and allowed to cool in the flask, it solidified to a glassy, brown solid, which partly dissolved when water was added, leaving a brown oily substance in the solution. The distillate was made up to 500 cc. and titrated in 25-cc. portions. On the basis of a coumarin titration each sample should have given a titration value of about 12.7–12.9 cc. of 0.1*N* potassium permanganate. Instead there was obtained a titre of 0.8 cc., thus indicating that only a small portion of the organic matter had passed over into the distillate. This distillate gave a red color with the diazonium reagent. The residue in the flask after addition of water was extracted with ether. The ether was colorless and gave no appreciable residue on evaporation. When, however, the aqueous liquid was made acid with concentrated hydrochloric acid, the color of the solution changed to a lighter brown; on extraction with ether the colored material dissolved in the ether layer. After evaporating the ether, a golden to brownish bitter oil was left. It did not have the sweetish honey-like odor of the crystalline melilotic acid. When the oil was dissolved in sodium carbonate the crimson color developed on the addition of the diazonium reagent. It was concluded that this was due to the presence of melilotic anhydride. Thus it is seen that if melilotic acid were present in the ether extract of sweet clover and the distillation carried out, a certain amount would be liable to distil over, and would interfere with the titration value for pure coumarin.

Samples (10 gm.) of air-dried sweet clover were extracted for 20 hr. with ether and subjected to Obermayer's distillation and titration, and also to colorimetric estimation. It was found that even after 20 hr. of extraction the odor of coumarin persisted in the residue. To estimate how much coumarin remained unextracted, colorimetric determinations were made on alcoholic extracts of the residue. Coumarin estimation by the colorimetric method was also made on the original plant material by comparing with a standard of coumarin in an alcoholic extract of western rye grass. The colorimetric estimations on the distillates were compared with pure coumarin standards. The mean results are shown in Table IV.

TABLE IV

COMPARISON OF OBERMAYER'S AND THE PROPOSED COLORIMETRIC DETERMINATION OF COUMARIN IN SWEET CLOVER

Material tested	Method used	Coumarin, %
1. Total plant material	Colorimetric on alcoholic extract	0.3103
2. Residue from ether extraction	Colorimetric on alcoholic extract	0.2014
3. Distillate from ether extraction	Colorimetric method	0.0965
4. Distillate from ether extraction	Obermayer permanganate titration	0.1856
	Addition of results of Methods 2 and 3	0.2979
	Subtraction of result of 2 from that of 1	0.1089

These results show that: (i) a considerable portion of the coumarin or melilotic acid was not extracted by the ether in 20 hr.; (ii) the Obermayer distillate contains substances that are reduced by permanganate and yet do not give the color produced by pure coumarin, *i.e.*, only a portion of the titration is accounted for by the oxidation of coumarin; (iii) there is close agreement between the amount of coumarin determined by the colorimetric method in the total plant material (0.3103%) and the sum (0.2979) of the amounts found in the distillate (0.0965%) and in the residue (0.2014%).

To an ether extract of the same sweet clover sample, 0.03 gm. of coumarin was added, the ether evaporated, and the mixture distilled. By the addition of 0.03 gm. of coumarin the percentage determinations should each be raised by 0.3%. The results (obtained both colorimetrically and by permanganate titration) are given in Table V.

TABLE V

VERIFICATION OF METHODS BY ADDITION OF PURE COUMARIN TO THE ETHER EXTRACT

Method	Coumarin, %		
	Observed	Previous determination	Increase in coumarin
By titration	0.4871	0.1856	0.3105
By colorimetric method	0.3935	0.0965	0.2970

It is seen that the added coumarin appears in both determinations. It appears therefore that the extra 0.0891% obtained by permanganate titration represents other substances which reduce the permanganate and give a fictitious value for the coumarin determination.

The foregoing observations lead to the conclusion that the colorimetric method for estimating coumarin and melilotic acid is reasonably accurate, and is not subject to some of the errors which occur in the Obermayer method. The colorimetric method is very much shorter and allows a large number of samples to be tested in a short time. The tentative outline of the test calls for an extraction of 1 gm. of material, but in practice the method can be applied to smaller amounts of green material and even to short sections of leaf or stem from a single plant. By application of this method, single growing plants may, without injury, be tested for selection purposes.

Application of Colorimetric Test to Sweet Clover Seed

The alcoholic extract of the seed is of a pale lemon color, which on addition of sodium carbonate turns a bright yellow. When the diazonium solution is added a brilliant crimson results if coumarin or melilotic acid is present. In this case also it was necessary to compare the colors obtained with standard coumarin solutions in an alfalfa seed extract or an extract of some other similar seed which contains no coumarin. As a tentative procedure it is suggested that a 1 gm. sample of the seed be crushed or ground and extracted by heating it to boiling with 25 cc. of 95% alcohol, the solution shaken for half an hour, water added to make up to 50 cc. and the solution again shaken for half an hour. After allowing the solution to stand for one hour, an aliquot of 5 cc. can be tested by the usual method. A series of standards of 5 cc. of alfalfa extract prepared in the same manner should have sufficient coumarin solution added to them (from 0.0001 to 0.0015 gm.) to give a range of concentrations from 0.1 to 1.5%. Then by matching the color developed in the sweet clover extract to the nearest alfalfa standard by eye, and making the final comparison in the colorimeter, closely replicable estimates can be made. Because of the brilliant golden color of the seed extract after treatment with sodium carbonate, it is not possible to match the crimson colors over a wide range in the colorimeter, and therefore a large number of alfalfa standards should be set up.

Conclusion

(i) With a pure coumarin solution the intensity of the colors obtained by coupling with diazotized *p*-nitraniline in alkaline solution under standard prescribed conditions is proportional to the concentrations of coumarin and can be matched in a colorimeter. (ii) With a pure coumarin solution the results obtained by colorimetric comparisons agree with the results obtained by Obermayer's permanganate titration method. (iii) If melilotic acid is present in the material used for Obermayer's distillation method, reliable quantitative determinations for coumarin cannot be made, owing to a partial distillation of the melilotic acid, which is reduced by permanganate. (iv) For practical work, the color produced by treating coumarin with the diazonium solutions is roughly equivalent to that obtained with the same amount of melilotic acid, and thus a combined determination of the coumarin and melilotic acid in sweet clover can be made. (v) In testing Obermayer's method for coumarin in sweet clover and checking it with the colorimetric method it was shown that— (a) an ether extraction of sweet clover material is not complete in 20 hr.; (b) the distillate obtained in the Obermayer method contains a substance which is oxidized by permanganate, but which will not give the crimson color due to coumarin or melilotic acid on treatment with the diazonium reagents, and therefore may be regarded as impurity in the distillate.

A tentative method is outlined for a coumarin–melilotic-acid determination on alcoholic extracts of sweet clover plants by comparison with standards set up with pure coumarin in alcoholic extracts of western rye or alfalfa plant

material. There is not yet sufficient proof to justify a claim that this procedure is anything but comparative, but knowing that with colorless solutions the method of coumarin determination is quantitative, sufficient reliance may be placed on the results obtained to make the test of practical importance to the plant breeder.

Further work should aim at the technical improvement of the method of extracting the coumarin from the plant material, with consequent improvement in the quantitative relations of this test for coumarin estimation.

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DECOMPOSITION AND MOVEMENT OF HERBICIDES IN SOILS, AND EFFECTS ON SOIL MICROBIOLOGICAL ACTIVITY AND SUBSEQUENT CROP GROWTH. PART II[1]

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Abstract

Further experiments at Edmonton to determine the effects on soils and on subsequent crop growth of copper sulphate, sodium chlorate, and sodium dichromate, are reported, together with new experiments with ammonium thiocyanate. The field plot experiments were limited to Edmonton black soil, but three typical Alberta soils, including Edmonton soil, were used in the laboratory experiments. Copper sulphate applied to a series of fallow plots in 1931 did not affect the yields of wheat on these plots significantly in 1932, or the yields of wheat, oats, rye and flax on these plots in 1933. The soil was not appreciably injured, even temporarily, by the copper sulphate. Sodium chlorate was applied to one series of fallow plots in 1930, and to another series in 1931. The effect of the heaviest applications (1300 lb. per acre) lasted for three years in one series, but serious injury to crops from such heavy applications did not last for more than two years in either series. Sodium dichromate was applied to a series of fallow plots and a series of wheat plots in 1932. It reduced the wheat yields very much in 1932, but did not reduce crop yields the following year in either series, as it decomposed and lost its toxicity in the soil rather quickly. Ammonium thiocyanate was applied to a series of fallow plots and a series of wheat plots in 1932, and it reduced the wheat yields even more than sodium dichromate in 1932. It retarded nitrification and did not decompose and lose its toxicity completely during the season of application, but even in the cases of the heavier applications (650 and 1300 lb. per acre) the toxic effect disappeared early in the following season. The total and straw yields of crops sown on these plots were generally increased by the lighter applications (160 and 325 lb. per acre) in 1933, but the grain yields were generally reduced by the heavier applications (650 and 1300 lb. per acre). Growth of certain annual weeds was considerably stimulated by this nitrogenous weed killer in 1933 and 1934, in the plots to which the heavier applications had been made. Laboratory experiments showed that the thiocyanate may be leached out of a soil with water; that it decomposes fairly rapidly in soils under favorable conditions of moisture and temperature and more rapidly in fertile soil rich in organic matter than in poorer soil; and that nitrification in soils is depressed for a time by the ammonium thiocyanate.

In Part I of these studies (3) experiments were reported concerning the residual effects of sulphuric acid, copper sulphate, sodium chlorate, barium chlorate and sodium dichromate applied as weed killers to certain Alberta soils. The present paper deals with further experiments with sodium chlorate, copper sulphate, sodium dichromate, and experiments with ammonium thiocyanate.

The field plot experiments were limited to Edmonton black soil, but for pot culture and other laboratory experiments the following three types of Alberta soil were generally used: Edmonton black, a fertile black loam, high in organic matter; Breton gray, a typical leached wooded soil; and Winterburn fine sandy loam, a soil of intermediate fertility. The field experiments were

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conducted on Belgravia field by permission of the Field Crops Department, University of Alberta. The general plan of the field plot experiments was shown in the publication previously referred to (3).

As a rule three concentrations of each chemical were used in the experiments. These cover the range used in practice fairly well. The concentrations are designated in this paper as Check or Untreated, and Concentrations A, B, and C. Table I shows the concentrations of the various chemicals used.

TABLE I

RATES OF APPLICATION OF CHEMICALS TO SOIL REPRESENTED BY TREATMENTS A, B, C, ETC.

	Check	A−		A		B		C		C+	
		Gm. per 100 gm.	Lb. per acre	Gm. per 100 gm.	Lb. per acre	Gm. per 100 gm.	Lb. per acre	Gm. per 100 gm.	Lb. per acre	Gm. per 100 gm.	Lb. per acre
H_2SO_4	None	—	—	.0024	30	.004	50	.008	100	—	—
$CuSO_4$	None	—	—	.0012	15	.0024	30	.0048	60	—	—
$NaClO_3$	None	—	—	.026	327	.052	654	.104	1308	—	—
$Na_2Cr_2O_7$	None	.013	163	.026	327	.052	654	.104	1308	.146	1838
$Ba(ClO_3)_2$	None	—	—	.039	489	.078	978	.156	1960	—	—
NH_4CNS	None	.013	163	.026	327	.052	654	.104	1308	—	—

Methods

Laboratory methods used in these experiments were described or referred to in Part I of these studies (3). Methods of making the following determinations were then given: Chlorate in soil extracts; nitrate in soil extracts in the presence or absence of chlorate; dichromate in soil extracts; carbon dioxide evolution in soil; bacteria and fungi in soil.

The method of determining thiocyanate in water extractions of soils treated with ammonium thiocyanate was not described in the previous publication. It is a modification of a method given by Treadwell and Hall (10, p. 601) for the determination of silver, as follows: Titrate 50 cc. portions of a one-to-five water extract of soil with standard silver nitrate (approximately 0.025 *N*) past the end point, using ferric ammonium alum, containing enough nitric acid to discharge the brown color of the salt, as the indicator. Then titrate back with standard ammonium thiocyanate to the red end point due to formation of ferric thiocyanate.

Results

Sodium Chlorate

Field plot crop yields showing residual effects of different applications of sodium chlorate are shown in Table II. The 1931 yields were given in Part I of these studies (3), but are included in this paper for direct comparison with the subsequent yields of these plots. Each yield represents the average of quadruplicate plots. The sodium chlorate was applied in July, 1930, and the wheat crop yields of the three following years are shown. It will be

TABLE II

EDMONTON FIELD PLOT CROP YIELDS, SHOWING RESIDUAL EFFECTS OF DIFFERENT APPLICATIONS OF SODIUM CHLORATE

$NaClO_3$ applied July, 1930		Wheat yield 1931	Wheat yield 1932	Wheat yield 1933
Check	Straw per acre, lb.	7760	2451	2345
	Grain per acre, bu.	50.1	26.1	17.1
A. Conc.	Straw per acre, lb.	1760	3301	2438
	Grain per acre, bu.	7.3	31.3	22.0
B. Conc.	Straw per acre, lb.	1020	2373	2399
	Grain per acre, bu.	1.7	24.3	21.8
C. Conc.	Straw per acre, lb.	340	2084	2465
	Grain per acre, bu.	0.02	20.2	20.4

observed that in 1931 growth was almost completely prevented by the largest application and greatly reduced by even the smallest application. In 1932 the wheat yields were not appreciably reduced except probably in the case of the C concentration (heaviest application) plots. In 1933 there was no reduction in yields of treated plots.

Another similar series of field plots was treated with sodium chlorate in the latter part of June, 1931; strips of wheat, oats, barley, and flax were sown across these plots in 1932, and wheat, oats, barley, rye, and flax in 1933. The yields are shown in Table III. It is apparent that the wheat yields were not reduced nearly as much the year following application of chlorate as in the case of the experiment started a year earlier. This is attributed mainly to a much higher rainfall during the late summer of 1931 than during the late summer of 1930, for, as pointed out in Part I of these studies (3) chlorates are readily leached down deeply into this soil by heavy

TABLE III

EDMONTON FIELD PLOT CROP YIELDS, SHOWING RESIDUAL EFFECTS OF DIFFERENT APPLICATIONS OF SODIUM CHLORATE

$NaClO_3$ applied June, 1931		Wheat yield		Oat yield		Barley yield		Rye yield	Flax yield	
		1932	1933	1932	1933	1932	1933	1933	1932	1933
Check	Straw per acre, lb.	4850	3744	6362	3026	5728	3131	3305	5186	2993
	Grain per acre, bu.	43.3	39.5	104.5	60.7	72.0	45.8	30.2	6.1	4.9
A. Conc.	Straw per acre, lb.	4330	3494	4588	2572	3160	2998	3464	3724	2572
	Grain per acre, bu.	39.6	37.5	90.9	59.2	30.6	35.8	38.1	13.4	7.9
B. Conc.	Straw per acre, lb.	2925	3025	4257	2132	2208	2305	2838	3368	2644
	Grain per acre, bu.	25.2	32.9	84.4	44.3	19.7	28.6	28.1	13.4	5.6
C. Conc.	Straw per acre, lb.	2497	2865	3347	1800	1136	2185	1745	2464	2513
	Grain per acre, bu.	23.2	31.1	62.3	42.3	10.4	26.2	12.8	13.3	5.1

rainfall. The total yields of oats, barley and flax were all decreased on the treated plots in 1932, the decreases being roughly proportional to the concentration of applied chlorate. In the case of flax, however, the heavier growth of straw on the untreated plots was accompanied by a smaller grain yield. In 1933, it will be observed, the wheat yields were still somewhat reduced by the heavier applications of 1931. The yields of oats, barley, and rye from the treated plots were decreased in 1933 (except in the one case of the rye plots which received the smallest applications of chlorate), the decrease being roughly proportional to the concentration of applied chlorate. In the case of flax, however, the differences between treated and untreated plots were not significant. It is possible, in this case, that the chlorate had leached down below the reach of the shallower rooted flax plants. Wheat alone was planted on these plots in 1934, but, owing to an outbreak of loose smut it was found necessary to plow down the crop about the middle of July. At this time there was no apparent effect of chlorate, but it is interesting to note that in the latter part of June the wheat leaves of the heaviest application plots were slightly burned, thus showing that some effect of the chlorate applied in 1931 was carried over into 1934. Before the middle of July this effect had disappeared, and it is unlikely that yields would have been appreciably reduced by the chlorate in 1934.

Copper Sulphate

Copper sulphate has been used successfully for the destruction of annual weeds. In Part I of these studies (3) its effect when applied to a growing crop of wheat is shown, and it was observed that soil nitrification is but slightly affected by its application.

A series of fallow plots was treated with copper sulphate on June 26, 1931. Yields of wheat in 1932, and yields of wheat, oats, barley, rye, and flax grown on these plots in 1933 are given in Table IV. Following the application of copper sulphate, in 1931, there was evidently no significant decrease in crop

TABLE IV

EDMONTON FIELD PLOT CROP YIELDS SHOWING RESIDUAL EFFECTS OF DIFFERENT APPLICATIONS OF COPPER SULPHATE

$CuSO_4$ applied June 26, 1931		Wheat yield 1932	Yields 1933				
			Wheat	Oats	Barley	Rye	Flax
Check	Straw per acre, lb.	4015	3345	3558	2905	3411	3838
	Grain per acre, bu.	35.5	30.6	77.2	40.2	35.7	4.8
A. Conc.	Straw per acre, lb.	4086	3225	3358	3225	3798	3691
	Grain per acre, bu.	34.2	28.0	76.2	46.3	34.5	5.5
B Conc.	Straw per acre, lb.	4013	3558	3478	3012	3691	3497
	Grain per acre, bu.	36.9	32.9	76.7	44.4	31.4	5.9
C Conc.	Straw per acre, lb.	3577	3651	3571	2932	3691	3561
	Grain per acre, bu.	28.9	30.2	81.1	41.6	31.2	4.5

yield in 1932 or 1933. The decrease of wheat yield in the 1932 C concentration plots was probably not significant, as the B concentration plots gave a somewhat higher yield of grain than the untreated plots.

Sodium Dichromate

Sodium dichromate has been used to some extent as a chemical weed killer. Water or nutrient solution experiments showed, as previously reported (3), that when placed in contact with germinating wheat seeds, and in contact with growing wheat plant roots, sodium dichromate is much more toxic to wheat than sodium chlorate in equal concentration. However, it was shown, in laboratory and greenhouse experiments, that sodium dichromate decomposes and loses its toxicity in the soil very rapidly, by comparison with sodium chlorate. While decomposing it has a depressing effect on numbers of soil micro-organisms, nitrification, and carbon dioxide production.

A series of growing wheat plots was sprayed with sodium dichromate on July 1, 1932. The wheat yields of that season, and the wheat, oats, barley, rye and flax yields of the following season are shown in Table V. It will be

TABLE V

EDMONTON FIELD PLOT CROP YIELDS SHOWING DIRECT AND RESIDUAL EFFECTS OF DIFFERENT APPLICATIONS OF SODIUM DICHROMATE

$Na_2Cr_2O_7$ applied July 1, 1932		Wheat yield 1932	Yields 1933				
			Wheat	Oats	Barley	Rye	Flax
Check	Straw per acre, lb.	2494	2079	1972	2132	2105	1897
	Grain per acre, bu.	29.5	21.3	54.1	25.5	25.7	11.8
A/2 Conc.	Straw per acre, lb.	1521	2612	3385	2772	2905	2292
	Grain per acre, bu.	14.2	25.8	65.1	37.7	26.2	10.5
A Conc.	Straw per acre, lb.	1379	2585	3171	3544	2852	2574
	Grain per acre, bu.	8.9	26.2	76.8	37.7	31.4	11.1
B Conc.	Straw per acre, lb.	791	3624	3171	3843	3065	2734
	Grain per acre, bu.	4.1	30.2	87.8	50.5	33.8	14.9

observed that the direct effect of spraying the wheat crop was pronounced, as even the lightest application reduced the grain yield to about one-half of that of the untreated plots. However, it will be observed that no injurious residual effect was carried over into the following season. The effect seemed rather beneficial as the yields, in 1933, were in nearly all cases higher following the heaviest application of sodium dichromate.

Sodium dichromate was applied to a series of fallow plots also, on July 1, 1932. This series included a heavier rate of application than the other series, as shown in Table VI. It seems clear that no injurious effect was carried

TABLE VI

EDMONTON FIELD PLOT CROP YIELDS SHOWING RESIDUAL EFFECTS OF DIFFERENT APPLICATIONS OF SODIUM DICHROMATE

$Na_2Cr_2O_7$ applied July 1, 1932		Yields 1933				
		Wheat	Oats	Barley	Rye	Flax
Check	Straw per acre, lb.	3892	3017	3705	4652	3545
	Grain per acre, bu.	30.2	57.5	39.4	44.3	2.6
A. Conc.	Straw per acre, lb.	4612	4433	5305	5238	4366
	Grain per acre, bu.	35.1	120.9	48.9	52.4	5.3
B. Conc.	Straw per acre, lb.	4012	4278	5132	5345	4465
	Grain per acre, bu.	34.6	116.4	46.9	52.6	4.9
C Conc.	Straw per acre, lb.	4065	4238	5118	4718	4630
	Grain per acre, bu.	34.4	111.3	46.6	43.3	3.0

over into the 1933 season, as the grain yields were rather higher on the heaviest or C concentration plots than on the check plots. The yield of the untreated oat plots is apparently irregular, as it is much lower than that of the treated oat plots, and the flax plot yields are somewhat irregular also.

AMMONIUM THIOCYANATE

Field Experiments

Field plot experiments like those carried out with sodium dichromate were also conducted with ammonium thiocyanate. A series of growing wheat plots was sprayed with ammonium thiocyanate early in July, 1932. The wheat yields of that season and the wheat, oats, barley, rye and flax yields of the following season are shown in Table VII.

TABLE VII

EDMONTON FIELD PLOT CROP YIELDS SHOWING DIRECT AND RESIDUAL EFFECTS OF DIFFERENT APPLICATIONS OF AMMONIUM THIOCYANATE

NH_4CNS applied July, 1932		Wheat yield 1932	Yields 1933				
			Wheat	Oats	Barley	Rye	Flax
Check	Straw per acre, lb.	3181	1546	2665	2825	2212	2095
	Grain per acre, bu.	27.4	16.0	64.3	33.3	23.3	8.8
A/2 Conc.	Straw per acre, lb.	738	2825	4424	4488	4904	4717
	Grain per acre, bu.	0.9	26.6	109.7	52.5	52.4	9.0
A Conc.	Straw per acre, lb.	781	3358	3891	4237	6108	4728
	Grain per acre, bu.	1.5	30.2	109.7	48.9	16.6	8.9
B Conc.	Straw per acre, lb.	805	1492	3465	3747	5863	4793
	Grain per acre, bu.	1.2	11.5	86.2	31.3	3.8	3.8

The direct effect of spraying the wheat crop was greater than in the case of sodium dichromate, as even the lightest application greatly reduced the straw yield, and reduced the wheat grain yield to a small fraction of that of the untreated plots.

The total crop yield in 1933 was increased in nearly all cases by the previous year's applications of ammonium thiocyanate, and this may be attributed, in part at least, to the nitrogen in this chemical. The beneficial effect upon

TABLE VIII

EDMONTON FIELD PLOT CROP YIELDS SHOWING RESIDUAL EFFECTS OF DIFFERENT APPLICATIONS OF AMMONIUM THIOCYANATE

NH_4CNS applied July, 1932		Yields 1933				
		Wheat	Oats	Barley	Rye	Flax
Check	Straw per acre, lb.	5889	4665	4492	6225	4044
	Grain per acre, bu.	38.6	108.7	42.2	52.1	7.5
A Conc.	Straw per acre, lb.	6964	5278	5038	7558	4785
	Grain per acre, bu.	42.1	95.6	33.9	53.6	1.7
B Conc.	Straw per acre, lb.	5674	5878	4438	8171	
	Grain per acre, bu.	28.0	57.2	29.7	15.9	
C Conc.	Straw per acre, lb.	4560	5085	5326	6377	
	Grain per acre, bu.	13.0	41.5	10.1	10.4	

total yield was generally greater following the lighter than following the heavier applications, and, in the cases of wheat, rye, and flax the grain yield was reduced by the heavier application. This indicated that complete decomposition of the thiocyanate had not taken place in the soil to which the heavier application had been made, and analyses of these plots reported in Table IX show that the thiocyanate did not decompose completely during

TABLE IX

UNDECOMPOSED THIOCYANATE REMAINING IN SOIL OF EDMONTON FIELD PLOTS TEN MONTHS AFTER APPLICATION

NH_4CNS treatment July, 1932		Sampling depth, ft., May 22, 1933	NH_4CNS recovered May 22, 1933	
			% of amount applied	lb. per acre
Check		0–1	00.0	00.0
		1–2	00.0	00.0
		2–3	00.0	00.0
A/2 Conc.	163 lb./acre	0–1	12.5	20.3
		1–2	00.0	00.0
		2–3	00.0	00.0
A Conc.	327 lb./acre	0–1	18.7	61.1
		1–2	00.0	00.0
		2–3	00.0	00.0
B Conc.	654 lb./acre	0–1	12.5	81.7
		1–2	3.1	20.3
		2–3	00.0	00.0

the season of application. There was some thiocyanate left in May, 1933, approximately ten months after the thiocyanate had been applied, especially in the soil to which the heavier applications had been made.

The color of the crops also showed that thiocyanate was still present in the soil in the early part of the 1933 growing season, as it was observed that the treated plot crops were yellowish, and that there were some withered leaf tips, especially in the plots to which the heaviest application had been made.

The heaviest application (B concentration) produced an increased growth of lamb's quarters, *Chenopodium album*, L., and ball mustard, *Neslia paniculata*, L., in 1933, especially in the flax plots. The growth of these annual weeds was apparently stimulated by the nitrogen in the ammonium thiocyanate.

Nitrates were determined in these plots in May, 1933, and the quantities of nitrate present in the soil to a depth of three feet are shown in Table X. These analyses indicate that nitrification was not prevented by the thiocyanate in the cases of the two lighter applications, but that nitrification

TABLE X

NITRATE NITROGEN IN SOIL OF EDMONTON FIELD PLOTS TREATED, TEN MONTHS PREVIOUSLY, WITH AMMONIUM THIOCYANATE

NH_4CNS treatment June 1932	Sampling depth, ft., May 22, 1933	Nitrate nitrogen p.p.m., May 22, 1933
Check	0–1	15.1
	1–2	4.2
	2–3	2.7
A/2. Conc. 163 lb./acre	0–1	25.0
	1–2	7.5
	2–3	2.9
A Conc. 327 lb./acre	0–1	24.8
	1–2	6.6
	2–3	5.5
B Conc. 654 lb./acre	0–1	15.1
	1–2	8.5
	2–3	7.3

was retarded in the case of the heaviest application or B concentration where the nitrogen added as ammonium thiocyanate would otherwise have been expected to produce the greatest accumulation of nitrates.

Ammonium thiocyanate was also applied to a series of fallow plots in early July, 1932. This series included a heavier rate of application than the other series, as shown in Table VIII. The B concentration reduced the grain yield considerably in 1933, and the C or highest concentration reduced the grain yield very much. The straw yield was not generally reduced by the heaviest application, and was in all cases increased by the lightest application. These

increases may be attributed to the nitrogen in this chemical. The decrease in grain yield of the B, and especially of the C concentration plots indicated that the thiocyanate had not decomposed completely in the plots to which the heavier applications had been made. This was indicated, also, by the fact that in the early part of the 1933 growing season the treated plot plants were yellowish in color, and, in certain cases, the leaves were withered. This effect seemed to disappear in the latter part of June and the beneficial effect of the nitrogen in the ammonium thiocyanate became apparent. The treated plot crops then became greener than the untreated, and the annual weeds present developed rapidly. The B and C concentration plots under wheat, oats, barley and rye were heavily infested with ball mustard, *Neslia paniculata*, L., and lamb's quarters, *Chenopodium album*, L., in 1933, and, under flax, even the A concentration plots were heavily infested with these weeds. The weeds, no doubt, had much to do with the reduced yields. The flax in the B and C concentration plots was almost completely choked out by these weeds. It seemed as though the growth of annual weeds was considerably stimulated by this nitrogenous weed killer, and that its concentration was not sufficient to destroy the weed seeds.

This stimulation of weed growth was again observed in 1934. Wheat was sown on the plots in May. In June and July it was noted that the wheat plant leaves on the treated plots were taller and darker green than the leaves in the untreated plots, especially in the case of the C concentration plots, to which the heaviest applications of ammonium thiocyanate had been made. This shows that the toxic effect of the thiocyanate had disappeared, and that the growth was stimulated by the nitrogen in this chemical. However, the growth of lamb's quarters, *Chenopodium album*, L., was also much greater on the treated plots, and this was especially true of the C concentration plots. Crop yields were not taken in 1934 as an outbreak of loose smut of wheat made it necessary to destroy the crop about the middle of July.

TABLE XI

AMMONIUM THIOCYANATE RECOVERED FROM SOILS BY LEACHING TEN DAYS AFTER IT HAD BEEN APPLIED (1000 GM. SOIL IN EACH CONTAINER)

NH_4CNS applied	NH_4CNS recovered	
	Gm.	% of that applied
Winterburn sandy loam		
Check	0.00	—
A/2 Conc. .13 gm.	0.049	37.7
A Conc. .26 gm.	0.127	48.8
B Conc. .52 gm.	0.272	52.4
Edmonton loam		
Check	0.00	—
A/2 Conc. .13 gm.	0.055	42.1
A Conc. .26 gm.	0.122	47.1
B Conc. .52 gm.	0.248	47.7
Breton wooded soil		
Check	0.00	—
A/2 Conc. .13 gm.	0.020	15.6
A Conc. .26 gm.	0.069	26.5
B Conc. .52 gm.	0.193	37.2

AMMONIUM THIOCYANATE

Laboratory Experiments

A laboratory experiment to compare the rates at which ammonium thiocyanate decomposes in different soils

was conducted with Winterburn sandy loam, Edmonton black loam, and Breton gray wooded loam, as shown in Table XI. The treated soils were kept at optimum moisture and room temperature for ten days. They were then leached with water and the quantities of ammonium thiocyanate present in the leachate were determined. It was observed that the thiocyanate decomposed rapidly under those favorable conditions in all three soils.

Another somewhat similar laboratory experiment was conducted with the same three soils. In this case, however, tumblers of treated soil, incubated at optimum moisture and room temperature, were removed for extraction and analysis every two weeks. Analyses of the water extractions are given in Table XII, and it will be observed that after six weeks incubation under

TABLE XII

AMMONIUM THIOCYANATE RECOVERED IN WATER EXTRACTIONS OF TREATED SOILS AFTER DIFFERENT PERIODS OF INCUBATION (TUMBLER EXPERIMENT)

Gm. NH_4CNS applied, per 100 gm. of soil	2 Weeks		4 Weeks		6 Weeks		8 Weeks	
	Gm.	% of that applied	Gm.	% of that applied	Gm.	% of that applied	Gm.	% of that applied
Edmonton black loam								
Check	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A/2 Conc. .013	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A Conc. .026	0.0032	12.5	0.0	0.0	0.0	0.0	0.0	0.0
B Conc. .052	0.0049	9.4	0.0	0.0	0.0	0.0	0.0	0.0
Winterburn sandy loam								
Check	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A/2 Conc. .013	0.0032	25.0	0.0	0.0	0.0	0.0	0.0	0.0
A Conc. .026	0.0065	25.0	0.0049	18.7	0.0	0.0	0.0	0.0
B Conc. .052	0.0106	20.4	0.0081	15.6	0.0	0.0	0.0	0.0
Breton gray wooded loam								
Check	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A/2 Conc. .013	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A Conc. .026	0.0065	25.0	0.0	0.0	0.0	0.0	0.0	0.0
B Conc. .052	0.0162	31.2	0.0081	15.6	0.0032	6.2	0.0	0.0

NOTE: *No NH_4CNS was recovered in the tenth and twelfth week incubations. The soil cultures were kept at optimum moisture and 28° C.*

favorable conditions, the thiocyanate had apparently decomposed completely in all except the sample of gray wooded soil to which the heaviest application of thiocyanate had been made. This experiment indicates that thiocyanate decomposes more rapidly in a fertile soil rich in organic matter than in poorer soils, as it disappeared most rapidly from the Edmonton black loam.

Determinations were made of nitrate as well as thiocyanate in these soil extracts, and the nitrate figures are shown in Table XIII. The quantities of nitrate were reduced by the thiocyanate in all three soils, especially at

TABLE XIII

EFFECT OF AMMONIUM THIOCYANATE ON SOIL NITRIFICATION (TUMBLER EXPERIMENT)

Treatment, gm. NH_4CNS per 100 gm. soil	Period of incubation					
	2 weeks	4 weeks	6 weeks	8 weeks	10 weeks	12 weeks
	Winterburn sandy loam: p.p.m. nitrate nitrogen					
Check	33	40	51	54	54	67
0.013	28	38	52	43	54	77
0.026	11	37	57	40	61	80
0.052	7	14	32	41	78	100
	Edmonton black loam; p.p.m. nitrate nitrogen					
Check	52	57	63	71	91	100
0.013	29	37	45	71	105	105
0.026	24	37	44	71	105	133
0.052	23	25	17	61	91	100
	Breton gray loam: p.p.m. nitrate nitrogen					
Check	6	20	28	20	62	81
0.013	5	4	9	10	15	43
0.026	trace	3	9	5	8	28
0.052	trace	trace	6	4	5	9

first. The heaviest application reduced nitrification for a period of eight weeks in all three soils, and, in the Breton gray loam, even the lightest application reduced the nitrification for at least twelve weeks. This was probably due to the toxicity of the thiocyanate, as the nitrogen in this chemical would otherwise be expected to produce a greater accumulation of nitrate.

Discussion

In the following discussion of the effects on soils and subsequent crop growth of chemicals used for weed control, some of the experiments reported in Part I of these studies (3) are referred to as well as the more recent observations.

The experiments show that sulphuric acid and copper sulphate have no injurious residual effect upon the soils studied. It is altogether likely that much larger quantities than those required to destroy annual weeds could be applied to these soils without causing injurious after-effects.

Sulphuric acid can be obtained at a lower cost than copper sulphate and another advantage of the acid is that the copper sulphate is relatively ineffective when rain falls within a few hours after spraying, whereas the sulphuric acid, by its quicker action, destroys the weeds effectively under the same conditions (2).

It is interesting to observe that although the sodium chlorate applied in 1931 did not injure the following year's crop of wheat nearly as much as the chlorate applied a year earlier, the toxic effect of the 1931 application lasted rather longer than that of the 1930 application. The reduction in yield of the second or 1933 crop of wheat following the 1931 application was greater than in the case of the corresponding crop following the 1930 application. The high rainfall of the late summer of 1931, which greatly reduced the injury of the 1931 application to the following year's crop, probably removed most of the chlorate remaining in the plots treated with sodium chlorate in 1930.

The presence of toxic quantities of chlorate in treated soils may be determined not only by its effects on crops in the field, but also by chemical analysis of the soils, and by its effect upon the growth of seedlings in small pots of the soil (3). The Winogradsky plaque culture method may be used also as a rapid microbiological test for the effect of chlorate in soils (4).

It has been pointed out by various workers that sodium chlorate is too expensive for use on large areas (7). The comparative cost and effectiveness of tillage and chlorates for the eradication of morning glory, Canada thistle and perennial sow thistle have been discussed recently by Tingey (9).

Sodium dichromate is highly toxic when first applied, or when placed in direct contact with the growing plant. It was much more toxic to wheat than an equal concentration of sodium chlorate in water cultures, and an application of about 160 lb. per acre (the lightest application) injured the growing wheat crop considerably when applied about the first of July. Sodium dichromate decomposes in the soil too rapidly to be of great value as a perennial weed killer, as it loses its toxicity, apparently, before it has reached and destroyed the underground root systems of many perennials. It might have value as a perennial weed killer when mixed with the less readily decomposable chlorate.

Ammonium thiocyanate applied to growing wheat reduced the yields even more than the corresponding applications of sodium dichromate, and, in contrast to the dichromate, the thiocyanate did not decompose and lose its toxicity completely during the season of application. However, it does decompose and lose its toxicity much more rapidly than sodium chlorate.

The effects of different applications of sodium chlorate and ammonium thiocyanate upon subsequent sowings of wheat in soil pot cultures in which the moisture content of the soil was carefully maintained have been reported recently by Bissey and Butler (1). They observed that with an application equivalent to 800 lb. per acre of sodium chlorate the toxicity persisted approximately two years, whereas the toxicity of an 800 lb. application of ammonium thiocyanate disappeared within two months, under these conditions of optimum moisture and no leaching. In spite of its relatively rapid decomposition in the soil it has been observed by Harvey (6) and other workers that ammonium thiocyanate is almost or quite as effective a destroyer of perennial weeds as sodium chlorate.

According to Harvey (6) the injurious after-effect of the chemical can be removed by applying a ferric salt, which reacts with the chemical to form non-toxic colloidal ferric thiocyanate. While this, of course, would add to the cost of the method, it offers a means for removal of the toxic action before it is desired to plant a crop.

Growth of certain common annual weeds was evidently stimulated by the nitrogen in the ammonium thiocyanate applied a year earlier, and its concentration was not sufficient to destroy the weed seeds, as a greater growth of lamb's quarters, *Chenopodium album*, L., and ball mustard, *Neslia paniculata*, L., occurred in the treated than in the untreated plots. The greatest growth of these weeds occurred in the plots which had in the previous season received the heaviest applications of ammonium thiocyanate. The flax crop was almost completely choked out by the weeds in these plots, for, as pointed out by Pavlychenko and Harrington (8), flax cannot compete with weeds as successfully as most of our common cereals.

If perennial weeds are successfully destroyed by a chemical, it may not be a serious objection if some annual weeds start to grow on the treated patches before the toxic effect of the chemical has entirely disappeared. However, the nitrogenous character of ammonium thiocyanate may be a disadvantage rather than an advantage, as subsequent growth of annual weeds on plots treated with sodium chlorate and sodium dichromate was not nearly as great as in the case of plots treated with the thiocyanate. It was observed by Grau (5) that when ammonium thiocyanate was used for the purpose of controlling turf weeds, it tended to encourage certain weeds.

It was pointed out in Part I of these studies (3) that when placed in contact with germinating wheat seeds, and in contact with growing wheat plant roots, in water culture experiments, sodium dichromate is much more toxic to wheat than sodium chlorate in equal concentration. According to Bissey and Butler (1) ammonium thiocyanate is similarly much more toxic than sodium chlorate in water cultures.

Acknowledgments

The authors wish to take this opportunity to express their appreciation to Dr. F. A. Wyatt, Head of the Department of Soils, for supervising the experiments during the year (1932-33) in which the senior author was absent from the University, to Mr. W. E. Bowser who was engaged in the earlier part of these experiments, and to Mr. P. Garrow for assistance in field plot work.

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MICROBIOLOGICAL STUDIES OF APPALACHIAN UPLAND PODSOL SOILS

I. EFFECTS OF PHYSICAL AND CHEMICAL TREATMENTS[1]

By P. H. H. Gray[2] and H. J. Atkinson[3]

Abstract

A study has been made of the effects of fertilizers and limestone, of fallowing, and of deep ploughing, upon certain aspects of microbial activity in representative Appalachian podsol soils at three farms in the Eastern Townships region of Quebec Province. The results show that the evolution of carbon dioxide and numbers of bacteria and actinomyces were not altered by any simple fertilizer applied annually for two years. Limestone, at the rate of six tons per acre, increased the numbers of micro-organisms during the two years of the experiment. Deep ploughing reduced both carbon dioxide and bacterial numbers in soils ordinarily ploughed to a normal depth.

Studies have also been made to determine the effects of some other chemical treatments upon the soil microflora. Carbon dioxide, bacterial numbers and nitrification of soil nitrogen were increased in field plots after treatment with calcium oxide, sodium carbonate and sodium hydroxide, alone or in combination; the effects lasted through two seasons.

The nature and distribution of podsol soils in Quebec Province have been discussed in previous papers (2, 4, 5). The high percentage of organic matter in these soils is a potential source of fertility, provided some means can be found of making its valuable food elements more readily available for plants. Since the decomposition of organic matter is largely due to microbial activity, treatments that stimulate the action of micro-organisms should help to increase soil fertility. The authors present in this paper the results of microbiological studies in field plots that have, during four years, received such treatments as might be expected to stimulate microbial activity.

A study was first made of the effects of soil treatments and fertilizers in common use, namely, ground limestone; nitrogenous, phosphatic and potassic fertilizers; deep ploughing in comparison with normal ploughing; and the effects of cultivated summerfallow. In the spring of 1931, sets of plots were laid out on three farms in the Eastern Townships region of Quebec; one in Brome County, one in Compton County, and one in Sherbrooke County. The location of these areas is shown in Fig. 1. In each case the soil was of the typical heavily leached podsol type, and had received no previous treatment except organic manure.

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FIG. 1. *Sketch map showing location of plots* (×) *in the Eastern Townships region of Quebec.*

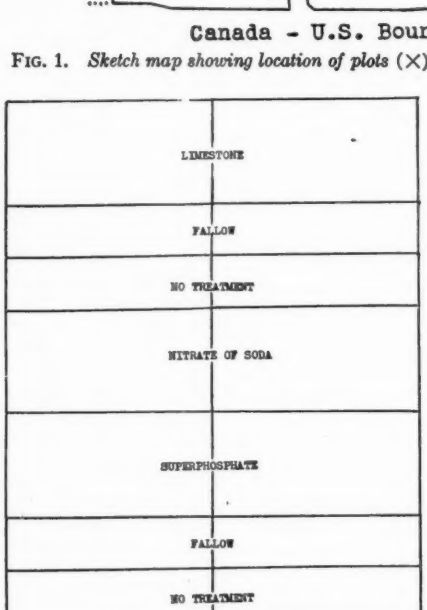

FIG. 2. *Plan of plots in experiments of 1931.*

An area of one acre was subdivided and treated according to the plan shown in Fig. 2. In one direction the area was divided into two equal parts, one-half being ploughed to a depth of 10–12 in., the other half to the customary depth, five or six inches. In the other direction the area was divided into six equal parts, as shown in the plan. Each treatment thus covered one-twelfth of an acre.

Oats were sown on the Brome County and Compton County plots, and corn on the Sherbrooke County plots.

The rates at which the treatments were applied per acre were as follows:—

Pulverized limestone (satisfying the lime requirement test by Jones' method)	6 tons
Nitrate of soda	200 lb.
Superphosphate (16%)	800 lb.
Muriate of potash	200 lb.

The chemicals were harrowed in immediately after application. The fallow plots were cultivated during the summer of 1931.

It was decided to limit the investigation to changes brought about in the soil itself by the treatments used. Representative soil samples for investigation were obtained by boring into the soil to a depth of six inches, by means of a piece of iron piping having an inside diameter of about one and three-quarter inches. When the sampling tool was withdrawn, the core of soil was removed by hammering on the outside of the pipe. Ten such samples, well distributed over the area, were taken from each one-twelfth acre plot and thoroughly mixed on a rubber sheet. A representative sample of about three pounds was taken from this. All samples were brought to the laboratory in small covered tins immediately after being taken and their examination was begun within 24 hr. of the time of sampling. Each set of plots was sampled four times during the growing season, at intervals of six weeks, the first samples being taken in each case before the application of the treatments.

The investigation was continued during 1932. The plots were ploughed in the fall of 1931, in the same way as had been done previously. In the spring of 1932 the applications of nitrate of soda, superphosphate, and muriate of potash were repeated. The plots that were fallowed during 1931 were cropped in 1932, Swede turnips being grown at all three places.

The biological determinations included the numbers of bacteria and actinomyces, the evolution of carbon dioxide, and the amount of nitrate nitrogen in the fresh samples, by the methods described in a previous paper (2).

Early in the work a test was made to determine the variation in biological factors within a single plot. For this purpose ten samples of four cores each were taken from each of two of the one-twelfth acre plots in Brome County; for the necessary comparison, a composite sample of ten single cores was made in the usual way at the same time. The ten samples and the composite sample from each plot were analyzed for carbon dioxide, and the numbers of bacteria were estimated.

The amounts of carbon dioxide and the numbers of micro-organisms found in these samples are shown in Table I.

TABLE I

CARBON DIOXIDE, BACTERIA AND ACTINOMYCES IN SOIL SAMPLES

	Carbon dioxide, mg. per 100 gm. per 100 hr.		Bacteria and actinomyces, millions per gm.	
	Average of 10 subsamples	Composite	Average of 10 subsamples	Composite
Plot 2	17.78 ± .743	17.52	16.59 ± .949	17.48
Plot 6	12.92 ± .627	12.63	13.60 ± 1.1296	15.54

It appeared from this test that the technique of sampling by means of ten single-core samples would be representative of biological activity in a plot.

It should be noted, however, that Plot 3 (no treatment, cropped) was divided into half plots distributed as shown. There was no appreciable difference between the analyses for each factor in these two smaller plots, in the experiment in Brome County. In the results to be considered below, this subdivision, and also that of Plot 2 (no treatment, fallow in 1931), may be a factor that would give different weight in an analysis of variance. A further limitation to accurate analysis must be observed in regard to the distribution of chemical treatments (arrangement of plots) at each place; these were not randomized within each acre area. The similarity of biological activity, however, at the three places, in regard both to levels and to direction of changes during two seasons, studies of which will form the subject matter of a second paper in this series, suggests that the results obtained from the plots receiving no chemical treatments can be accepted as representing reliable values.

Limestone was the only treatment that appeared to affect the biological activity in the plots. The results in regard to bacterial numbers, carbon dioxide, and nitrates in fresh samples, are shown in Table II, in which the plots treated with limestone are compared with the untreated cropped plots. In view of the fact that seasonal changes of numbers between the first and the second samples might have been masked by the treatment, the values shown are the averages of the three samples taken after treatment in the first year, and of the four samples taken in the second year. In regard to carbon dioxide, the results for the second year only are shown, since the chemical action of the limestone, from which carbon dioxide was freed by soil acids, obviously masked any differences due to biological action for at least twelve weeks after

TABLE II

MICROBIOLOGICAL RESULTS IN THE EXPERIMENTS OF 1931

Locality	Brome		Compton		Sherbrooke	
Ploughing	Normal	Deep	Normal	Deep	Normal	Deep
			Results in 1931			
Numbers of bacteria and actinomyces; millions per gm.						
Limestone	14.15	18.30	20.11	20.12	26.75	22.07
No treatment	13.98	11.09	10.42	12.94	14.71	10.72
			Results in 1932			
Numbers of bacteria and actinomyces; millions per gm.						
Limestone	13.54	15.62	15.03	12.66	37.68	19.89
No treatment	13.29	10.51	8.58	10.25	18.94	14.60
Carbon dioxide, mg. per 100 gm. per 100 hr.						
Limestone	32.25	39.55	49.03	36.60	80.05	53.90
No treatment	27.05	15.43	22.03	21.35	50.68	32.70
Nitrate nitrogen, parts per million						
Limestone	12.72	9.81	43.09	30.91	29.36	31.91
No treatment	10.63	8.21	30.25	26.92	24.60	32.99

treatment. Nitrate nitrogen was used up rapidly during the first year, and no values were therefore available in the later samples, except in those from the fallow plots; the values shown in Table II are the averages of the four samples taken in 1932, in all of which nitrates were found to be present.

It is clear from the results shown in Table II that limestone increased activity in these acid soils, and that the effects lasted through a second season.

In view of the fact that bacterial numbers are known to fluctuate extensively in untreated field soil, it did not seem reasonable to determine the degree of significance of the effects produced by limestone, simply on the basis of deviation of values from the first samples in the plots under consideration. An analysis of variance (1) was therefore made, using all of the values obtained from all of the plots. Bearing in mind the limitations mentioned above in respect to the arrangement of the plots, and also the evident similarity of the results obtained at the three places, the analysis has been used to determine also the significance of the effects of the two depths of ploughing. The analysis takes into consideration the distribution of the areas in widely separated localities, the two seasons, and the four periods of sampling at intervals of six weeks during two seasons, and also interactions between any two such factors as appeared to be important sources of difference, namely, treatments, ploughing, and places.

The analysis in Table III shows on a basis of probability, ($P = .05$), that the limestone treatment alone was effective in increasing bacterial numbers. There appears also to be a significant reduction in numbers through ploughing to a greater depth than is normally the practice. Similar results were given by the analysis of the values for carbon dioxide; deep ploughing significantly depressed biological activity, and no differences were found between treatments (exclusive of limestone).

TABLE III

ANALYSIS OF VARIANCE FOR MICRO-ORGANISMS AND FOR CARBON DIOXIDE
(Experiments of 1931)

	Micro-organisms		Carbon dioxide	
	Degrees of freedom	Sums of squares	Degrees of freedom	Sums of squares
Treatments	5	1601.16	4	1104.75
Ploughing	1	148.06	1	1138.40
Places	2	1636.13	2	5579.70
Years	1	56.39	1	359.91
Sampling	3	252.89	3	1008.28
Interactions:—				
Treatments × ploughing	5	119.88	4	239.40
Treatments × places	10	504.35	8	502.07
Ploughing × places	2	334.19	2	966.75
Residual	258	6330.62	214	20006.86
Total	287	10983.67	239	30906.12
Mean	14.021 ± 1.152		28.85 ± .624	
Standard deviation	4.953 ± .206		9.669 ± .441	

TABLE III—*Concluded*

MEANS

Treatments			Ploughing		
	Micro-organisms	Carbon dioxide		Micro-organisms	Carbon dioxide
Limestone	18.894		Normal	14.738	31.03
Fallow	11.548	24.86	Deep	13.304	26.67
Check	12.509	29.42			
Nitrogen	14.422	30.96			
Phosphorus	13.048	30.35			
Potash	13.705	28.65			
Difference required for significance	3.433	6.116		1.114	2.446

In searching for a cause for reduction of microbial activity brought about by deep ploughing, two possible factors have to be considered. The reduction found in the first samples must be due, probably solely, to dilution of the upper layer of soil with a proportionate amount of soil from the lower layer, in which the number of micro-organisms is much less than in the well tilled upper layer, a fact long known to all students of soil microbiology. It may not however be quite clear why this mechanical reduction should persist throughout two seasons, especially in view of the fact that the deeply ploughed half-acres of land were again turned over to the same depth in the second year, thus, presumably, effecting a more thorough mixing of the organic matter from which the micro-organisms obtained their material for energy and growth. With the data at present available it seems feasible to postulate that the reduced level of activity in the deeply ploughed plots in the second year can definitely be ascribed to the cultivation and not to changes induced by season. It may be stated that with both deep and shallow ploughing, biological activity in the second year was considerably less in the Brome and Compton soils, but considerably higher in the Sherbrooke soil; differences in bacterial numbers were in the same direction in all cases, but of a smaller order of difference; in spite of these irregular changes from one year to the next, deep ploughing, as has been shown, was a significant factor.

In addition to mechanical dilution, there is the factor of nutrition. In this case, the only available data regarding the nutrition of the organisms is contained in chemical analyses made from the first samples taken before the treatments were applied. In this connection, it seemed useful to determine whether or not the constituents analyzed were similarly reduced by deep ploughing. It was found that both carbon and nitrogen were thus mechanically diluted to a significant degree. In regard to mineral nutrients, there was a definite reduction in the amount of calcium, but magnesium and phosphorus were altered irregularly; the total of these differences was probably negligible. Whatever effects deep ploughing had on the alteration in the proportion of nutrients, it cannot be claimed that the differences found had

any biological significance; such effects, if any, would only be felt after a considerably longer time than two seasons.

It will be noted, in Table II, that numbers of micro-organisms in the deeply ploughed untreated plot in Compton County were higher than those in the normally ploughed plot, in both years of the experiment. A difference of a similar order was obtained after summarizing the results from all of the plots at this farm. The results were similar in the case of the analyses for carbon dioxide, excluding the plots treated with limestone. The actual mean values for these two biological factors are shown in Table IV.

TABLE IV

Ploughing	Micro-organisms, millions per gm.			Carbon dioxide, mg. per 100 gm. per 100 hr.		
	Brome	Compton	Sherbrooke	Brome	Compton	Sherbrooke
Normal	13.59	10.95	19.42	27.06	26.86	39.17
Deep	12.73	12.04	15.14	20.41	28.15	31.46

It appears to be worth recording that it had been the practice at the Compton County farm for many years to plough to a greater depth than was customary elsewhere. After a considerable number of seasons, therefore, deep ploughing of these soils may prove beneficial to microbial activities.

During the winter of 1931-32, chemical investigations in the Macdonald College laboratories (6) had shown that certain strong alkali treatments were effective in increasing the amount of soluble organic matter. It was thought that the use of these treatments in field plots should be studied and their effect on the activity of micro-organisms determined. For this purpose small plots were laid out in the spring of 1932 at each of the three farms, and close to the 1931 plots. Each plot was circular in shape, and enclosed an area of one hundred square feet (radius = 5.64 feet). Twelve such plots were installed at each of the three farms, randomized in two rows of six plots each, and the centres of the plots were exactly thirteen feet apart. The treatments used are shown in Table V. These treatments were applied to the surface of the soil in the spring of 1932, and harrowed in. They were not repeated in the succeeding year. Oats, seeded down with a timothy and clover mixture, was the crop grown at the three places.

Samples of four cores each were taken from each circular plot at intervals of six weeks during the season, at the same time as samples were taken from the plots laid down in the previous year, and the same biological analyses were made in the laboratory. In view of the fact that the areas in Brome County and Sherbrooke County had been previously treated with coarsely ground limestone, unevenly spread, it was decided after one season to limit attention to the plots in Compton County, which had not previously received such treatment. For this reason the results shown in Table V refer only to the plots at the latter place.

TABLE V

TREATMENTS, AND RESULTS OBTAINED, IN THE COMPTON COUNTY EXPERIMENT OF 1932

Plot No.	Treatment	Rate per acre	1932			1933	
			Carbon dioxide[1]	Micro-organisms[1]	Nitrate nitrogen[2]	Carbon dioxide[3]	Micro-organisms[3]
1	Na_2CO_3	1000 lb.	105	267	139	104	147
2	CaO	Lime requirement	236	350	298	156	156
3	Na_2CO_3	1000 lb.	184	423	565	139	210
	CaO	Lime requirement					
4	NaOH	400 lb.	153	336	273	111	151
5	Nil	Nil	100	100	100	100	100
6	NaOH	400 lb.	207	274	361	169	157
	CaO	Lime requirement					
7	CaO	Double lime requirement	202	422	285	167	186
8	Na_2CO_3	500 lb.	121	297	258	110	131
9	NaOH	200 lb.	131	183	trace	113	102
10	Na_2CO_3	500 lb.	201	305	344	146	147
	CaO	Lime requirement					
11	NaOH	200 lb.	174	297	170	120	160
		Lime requirement					
12	Na_2CO_3	2000 lb.	153	1355	721	77	396
	Value required for significance		109	120	118		

[1] *Averages of three samplings;* [2] *In fresh samples six weeks after treatment;* [3] *Averages of four samplings.*

In order to obtain a basis for an estimate of the significance of any changes due to treatment within the area enclosing the twelve plots, samples of four cores each were taken from outside the circular area of each of six plots, chosen at random, before the treatments were applied. It was found that a difference of over 9% in the amount of carbon dioxide would be required for significance. In the case of bacterial numbers, it unfortunately proved impossible to determine the limit for significance with the six samples collected at this time, on account of low numbers of colonies and their irregular dispersion on the plates. In view of the comparative variability of carbon dioxide and bacteria in the previous test it seemed justifiable to postulate 20% as the limit. The limit of significance for nitrate nitrogen was found to be 18%.

In reference to the results, as shown in Table V; it appears that every treatment was effective in stimulating biological activity in the first season, with the exception of sodium carbonate at the rate of 1000 lb. per acre, which did not stimulate the production of carbon dioxide. The presence of only a trace of nitrate nitrogen in the sample from plot No. 9 (200 lb. of sodium hydroxide per acre), can only be ascribed to some unknown accidental cause or causes.

The great increase of bacteria in plot No. 12, with 2000 lb. of sodium carbonate, was accompanied by the destruction of all higher plants in this plot.

During the second season the results showed that the treatments were still effective though in all cases to a lower degree in comparison with the untreated plot. In one case only, that of carbon dioxide in plot No. 12, activity was definitely reduced to a value significantly below that of the untreated plot No. 5. Nitrate nitrogen was not determined in the fresh samples during 1933.

The results can be more easily compared by combining the values obtained in the two seasons and expressing them in the form of graphs, as shown in Figs. 3–5. The results have been arranged in order of magnitude, after halving the sums of the proportional values for carbon dioxide and numbers

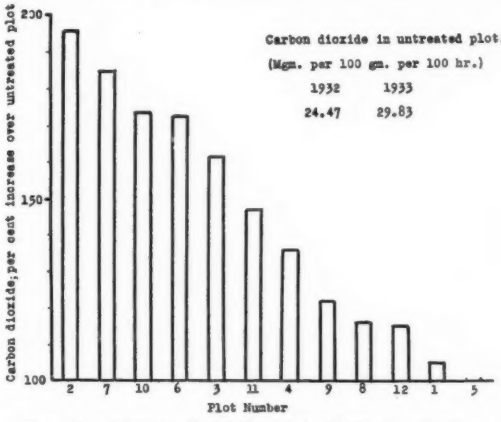
Carbon dioxide in untreated plot.
(Mgm. per 100 gm. per 100 hr.)
1932 1933
24.47 29.83
Carbon dioxide, per cent increase over untreated plot
200
150
100
2 7 10 6 3 11 4 9 8 12 1 5
Plot Number

FIG. 3. *Comparative effects of alkali treatments on biological activity in podsol soil.* [*Mean proportional values for two seasons; untreated plot (No. 5) = 100*].

of micro-organisms. The values for nitrate nitrogen remained as quoted in Table V. It will be seen that, with the exception of carbon dioxide in plot No. 12, there is a close agreement as to the comparative effects of the treatments, in the three biological factors studied. It is interesting to note that in the results for the evolution of carbon dioxide the order of effectiveness was calcium oxide, sodium hydroxide, and sodium carbonate, though sodium carbonate with calcium oxide takes a high place.

It is probably not possible to make a close comparison of these results with those of other workers who have reported the effects of similar treatments. The rates of application reported here were calculated on the basis of one acre, but the action of the compounds was probably largely on the soil within the top two or three inches, and may thus have been more concentrated than the figures would imply. For example, with sodium hydroxide, the organic matter on the top of the soil was burned brown. No tests have so far been made to determine the effects at different levels in the soil. It is clear however that they have stimulated the activity of the bacteria commonly associated in the destruction of organic matter, and also the oxidation of the nitrogen compounds in the latter.

In no case was evidence obtained that the calcium oxide induced partial sterilization; at least there was no evidence of any reduction in the biological factors six weeks after its application; such action may have occurred in close contact with the lime, until the excess of oxide had been converted into carbonate. On a basis of lime requirement the calculated amount of calcium

oxide, as burned lime applied, was 4000 lb. per acre; this was equivalent to 0.2%, assuming the lime to have been mixed into the soil. In laboratory experiments with caustic lime, Hutchinson (3) showed that 0.1% had no effect on the soil micro-organisms, while 0.5% caused a great increase in bacteria in soils containing from 2 to 3% of calcium carbonate.

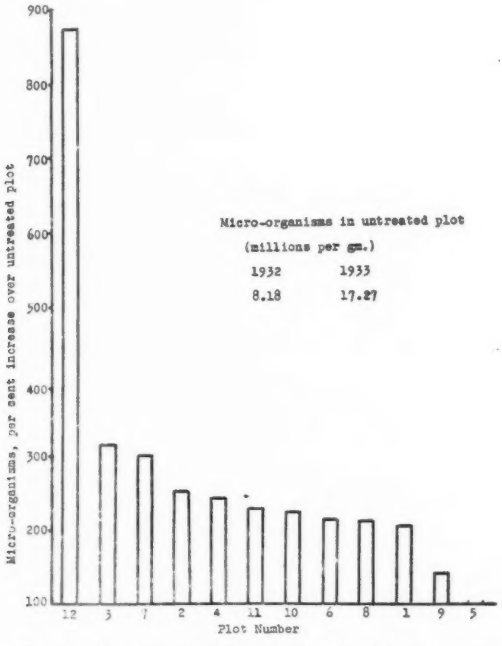

FIG. 4. *Comparative effects of alkali treatments on numbers of micro-organisms in podsol soil.* [*Mean proportional values for two seasons; untreated plot (No. 5) = 100.*]

The results obtained in the field plots with sodium carbonate and the other soil amendments suggested the value of such chemicals for studies on crop yield in these soils. In the spring of 1933 a duplicated set of plots, each $\frac{1}{100}$ acre in area, was laid out on the Compton County farm. The treatments included, among others, those that were used in the small circular plots laid down in the previous year. In view of the possible importance of sodium carbonate, and also since it is the experience of farmers in the district that unleached wood ashes are beneficial to plant growth, one of the additional treatments comprised a mixture of chemicals, approximating the fertilizing components of high grade wood ashes. The composition was as follows, on a basis of lb. per acre: calcium carbonate 6000, sodium carbonate 600, superphosphate 250, muriate of potash 200. The high amount of sodium carbonate in this mixture, relative to its amount in natural wood ashes, will be remarked.

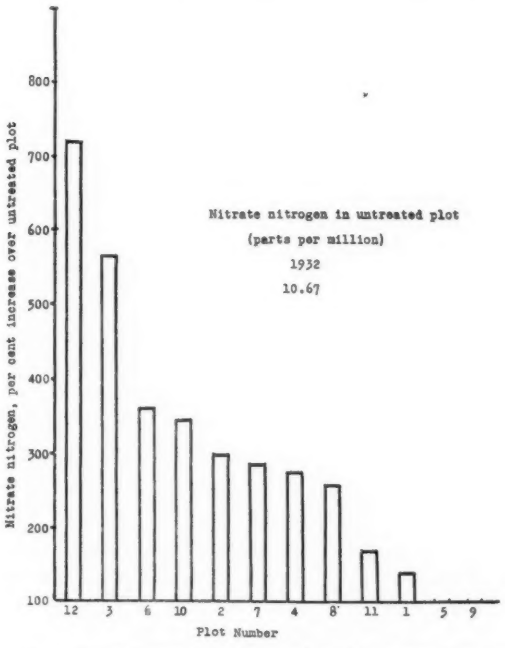

FIG. 5. *Comparative effects of alkali treatments on nitrification in podsol soil.* [*Proportional values for season of 1932; untreated plot (No. 5) = 100.*]

Samples were taken during 1934, to study the effect of limestone, sodium carbonate, and the "synthetic wood ashes" upon the numbers of micro-organisms one year after treatment. On the dates shown in Table VI, samples of five cores were taken from two similarly treated plots, one in each half-acre, of each of the three treatments selected for study, and from two of the untreated plots. The numbers of micro-organisms were determined by

TABLE VI

NUMBERS OF MICRO-ORGANISMS IN PLOTS OF 1933

Treatment and rate, lb. per acre	Sodium carbonate, 1000	Limestone, 6000	"Wood ashes", 7050	No treatment
Method of counting and date	Micro-organisms, per gm. dry soil			
Plate May 12, 1934	110,160,000	45,055,000	41,595,000	27,590,000
Direct May 12, 1934	2,824,000,000	2,323,000,000	2,024,000,000	1,740,000,000
Plate June 18, 1934	46,400,000	33,805,000	31,900,000	18,605,000

the usual plating method, and also by means of the ratio method in soil films (7). In the table each value represents the mean of the two plots in each case.

It is necessary to point out that the original counts of June 18, in respect of the fresh samples from the sodium carbonate and "synthetic wood ashes" plots, were not reliable; the samples were replated later, and the values shown are those calculated by ratio to a similarly replated sample from the control plots; the true proportionate values are therefore unknown. It is interesting to note that the total cell counts, while not directly proportional to the plate counts, show that the differences between treatments fall into the same order.

The results with limestone and sodium carbonate confirm those found previously, in that the effects of these treatments have lasted for two seasons; the sodium carbonate also, appears to be much superior to limestone; the results with the latter treatment also appear to be comparable with those of the same treatment in the plots of 1931, one year after its application.

Acknowledgment

The authors wish to acknowledge the assistance of Messrs. C. B. Taylor, E. P. Grant and H. R. Hudston who have been employed as technical assistants in this work at different times.

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SOME PREDATORS OF THE EUROPEAN RED MITE, *PARATETRANYCHUS PILOSUS* C. & F., IN NOVA SCOTIA[1]

BY F. C. GILLIATT[2]

Abstract

Predators are the most important natural enemies of the European red mite. Notes are given on the life history and habits of the following predaceous enemies of this mite, *Seiulus pomi* Parrott; *Diaphnidia pellucida* Uhl.; *Diaphnidia capitata* Van D.; *Hyaliodes vitripennis* Say; *Stethorus punctum* Leconte; *Plagiognathus obscurus* Uhl.; *Camptobrochis nebulosus* Uhl.; *Anystis agilis* Banks; *Campylomma verbasci* Mey., and an unidentified species of Syrphidae.

During the summer of 1932 a study was made of the factors pertaining to the natural control of the European red mite in Nova Scotia. At the outset, the writer wishes to state that such a study leads one into byways and side issues, from which it is difficult to become extricated, and in the final analysis the conclusions may not be too clearly defined.

As far as can be determined, the most important natural control factors, relating to this orchard pest, are the numerous predaceous enemies. At no time were internal parasites observed. The known predators are varied and include species representing one order of the class Arachnida and four orders of the class Hexapoda. Those belonging to the first class are two species of the order Acarina, *viz.*:—*Seiulus pomi* Parrott and *Anystis agilis* Banks. Of the second class there are six species of the order Hemiptera, *viz.*:—*Diaphnidia pellucida* Uhl., *Diaphnidia capitata* Van D., *Hyaliodes vitripennis* Say, *Plagiognathus obscurus* Uhl., *Camptobrochis nebulosus* Uhl., *Campylomma verbasci* Mey. (all Miridae). There was a species of Coleoptera, *Stethorus punctum* Lec. The Diptera represented was an unidentified species of the family Syrphidae. There was one unidentified species of the order Thysanoptera.

The more important of these predators were so difficult to rear under controlled conditions that all such work was largely abandoned. No doubt they could have been artificially reared by devising special technique, but this would have rendered it impossible to deal at any time with more than a small number, thus limiting the viewpoint in general. The results of the investigation, therefore, are largely based on field observations, supplemented to some extent by certain artificial control work using large field cages.

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Contribution from the Dominion Entomological Laboratory, Annapolis Royal, Nova Scotia, Canada.
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In the orchards examined there were always one or more species of predators feeding upon the European red mite. In some instances the predators were apparently few in number, while in many others they were numerous. It must be emphasized that it was not a simple matter in all instances to evaluate the various species of predators as far as numbers were concerned, for the reason that many are very minute and not readily observed without frequent, close and painstaking observations. Further, the various predators do not all appear at the same period during the summer. One may be disappearing when another is becoming abundant. There were periods between the generations of the predaceous mite *Seiulus pomi* Parrott when but few of the active forms were present, particularly during the first half of the season before there was any overlapping of the broods. As a result, in many instances, there were gaps during the season when predators were apparently not numerous and even difficult to find. When these gaps occur for any extended period the pest rapidly increases in numbers owing to its great powers of reproduction. It is not likely that any one of the predators, acting alone, will bring about a full measure of control of the mites. Certainly the best results are quite apparent where two or more are present. This, it might be stated, is usually what occurs and there is, as a rule, a very definite increase of all the predators as soon as the mites begin to appear in large numbers.

The writer does not wish to infer that there are no other natural control factors except predators. There doubtless are others, and this was considered when the decline of the pest did not seem to be consistent with the number of predaceous enemies present. It may be further stated that this was the most confusing part of the investigation, and, at the close of the season, was not entirely solved to the satisfaction of the writer.

There is another point which must now be considered. At certain stages in the lives of many of the predators large numbers are killed by the various sprays ordinarily applied for the control of fungus diseases and insect pests. This is described later in detail, but in a general way it may be mentioned that all the Miridae in the nymphal stages are quickly killed with nicotine spray; oils and some of the ordinary fungicides seem to affect seriously the number of *Seiulus pomi* Parrott, particularly in the immature and adult stages. This explains the rapid increase of the European red mite after application of oils.

Again, lime-sulphur sprays have a repressing effect upon the European red mite and such sprays ordinarily prevent this pest from assuming outbreak form. On the other hand no such results are in evidence following the use of Bordeaux mixture. In fact it has been shown that one of the most important predators, *Seiulus pomi* Parrott, was seriously reduced in numbers by Bordeaux spray. When a grower changes from a sulphur spray program to one of Bordeaux mixture, the European red mite frequently assumes outbreak form at the end of the first season following the use of Bordeaux spray. The only explanation that can be given is that up to the time of the change, the sulphur sprays kept the red mite under control, and with the change to Bordeaux mixture the controlling factor was removed, with the result that the mite

increased more rapidly than the predaceous enemies were capable of doing. In the orchards under special observation, the population of European red mite has been greatest on those areas treated with Bordeaux mixture, but orchards treated with sulphur sprays are, nevertheless, frequently subject to severe infestation.

All this, however, may be somewhat modified by various environmental conditions. The mite flourishes best in vigorous orchards and poorly cared for orchards of low vigor are not likely to show outbreaks of great degree. Again, when there are solid blocks of susceptible varieties, outbreaks are more liable to develop.

As a summary, the many predators must be considered in the light of a common enemy to the European red mite, a natural balance being established under normal environment. When there is interference with the factors of natural control there follows a rise in numbers of the pest which may end in destructive outbreaks.

Sprayed and Untreated Apple Trees in Relation to Prevalence of European Red Mite

It has been observed generally, that the European red mite appeared to increase in orchards sprayed with Bordeaux mixture in comparison with those treated with sulphur sprays of various kinds, or even in untreated orchards. All sulphur sprays are known to have a repressing influence upon red mite, while Bordeaux mixture does not have this effect. It also appears that natural control is influenced in some way by the use of Bordeaux mixture, probably as a result of the destruction or repulsion of the various predators that attack the mites. In England the European red mite has also been observed to increase more rapidly in Bordeaux sprayed orchards in comparison with those not so treated, and the increase has been attributed to a detrimental effect of the Bordeaux upon some natural control factor.

For the purpose of making regular counts of red mite throughout the season of 1931, an experimental orchard that had been treated with various standard sprays, was selected. Each plot in this orchard had received the same treatment for the preceding three years and a fair degree of European red mite infestation had been present for some time. The variety was Golden Russet, one of those susceptible to attack. There were five plots, four of which were treated with standard sprays and one check as follows: Bordeaux; lime-sulphur; lime-sulphur–iron sulphate mixture; lime-sulphur–aluminium sulphate mixture, and check plots. Counts of red mite were made every two weeks, or as near such dates as convenient. All counts were based on the number of mites, including all stages, per hundred leaves.

Table I fully explains the results of these counts. It will be observed that the Bordeaux treated plot at all times led the list in number of mites, by a wide margin. Early in the season lime-sulphur–iron sulphate mixture gave the least number of mites, but its place was soon taken by the straight lime-sulphur. Regarding the check, a word of explanation should be made. The

TABLE I

EUROPEAN RED MITE COUNTS FROM STANDARD SPRAY PLOTS, BERWICK, N.S., 1931

Date	Spray	Number of mites per 100 leaves			
		Adults	Eggs	Immature forms	Total, all stages
May 29	Bordeaux	0	0	169	169
29	Lime-sulphur	0	0	54	54
29	Lime-sulphur–iron sulphate	0	0	16	16
29	Lime-sulphur–alum. sulphate	0	0	41	41
29	Check	6	0	78	84
June 12	Bordeaux	29	1131	0	1160
12	Lime-sulphur	4	153	0	157
12	Lime-sulphur–iron sulphate	0	49	0	49
12	Lime-sulphur–alum. sulphate	3	124	0	127
12	Check	15	395	0	410
June 25	Bordeaux	7	422	732	1161
25	Lime-sulphur	0	12	4	16
25	Lime-sulphur–iron sulphate	0	42	9	51
25	Lime-sulphur–alum. sulphate	1	40	43	84
25	Check	6	215	166	387
July 9	Bordeaux	84	2011	15	2110
9	Lime-sulphur	5	174	2	181
9	Lime-sulphur–iron sulphate	7	70	0	77
9	Lime-sulphur–alum. sulphate	19	181	12	212
9	Check	84	1292	88	1464
July 28	Bordeaux	284	1558	310	2152
28	Lime-sulphur	15	32	20	67
28	Lime-sulphur–iron sulphate	15	127	10	152
28	Lime-sulphur–alum. sulphate	46	113	54	213
28	Check	73	298	86	457
Aug. 12	Bordeaux	130	2262	920	3312
12	Lime-sulphur	32	432	78	542
12	Lime-sulphur–iron sulphate	56	346	72	474
12	Lime-sulphur–alum. sulphate	42	486	192	720
12	Check	48	880	270	1198
Aug. 31	Bordeaux	220	2644	502	3366
31	Lime-sulphur	20	114	20	154
31	Lime-sulphur–iron sulphate	56	1038	110	1204
31	Lime-sulphur–alum. sulphate	34	488	48	570
31	Check	28	230	46	304
Sept. 16	Bordeaux	394	86	626	1106
16	Lime-sulphur	50	38	92	180
16	Lime-sulphur–iron sulphate	182	38	300	520
16	Lime-sulphur–alum. sulphate	38	22	120	180
16	Check	12	14	18	44
Sept. 29	Bordeaux	216	44	148	408
29	Lime-sulphur	54	0	4	58
29	Lime-sulphur–iron sulphate	184	10	80	274
29	Lime-sulphur–alum. sulphate	62	4	32	98
29	Check	26	0	6	32

TABLE I—(*Concluded*)

EUROPEAN RED MITE COUNTS FROM STANDARD SPRAY PLOTS, BERWICK, N.S., 1931—*Concluded*

Date	Spray	Number of mites per 100 leaves			
		Adults	Eggs	Immature forms	Total, all stages
Oct. 19	Bordeaux	64	0	8	72
19	Lime-sulphur	26	0	2	28
19	Lime-sulphur–iron sulphate	24	2	1	27
19	Lime-sulphur–alum. sulphate	24	5	2	31
19	Check	0	0	0	0
Nov. 3	Bordeaux	80	0	4	84
3	Lime-sulphur	36	0	0	36
3	Lime-sulphur–iron sulphate	38	0	0	38
3	Lime-sulphur–alum. sulphate	34	0	0	34
3	No leaves on check plots				
		Winter eggs on limbs			
Nov. 12	Bordeaux		3035		3035
12	Lime-sulphur		187		187
12	Lime-sulphur–iron sulphate		658		658
12	Lime-sulphur–alum. sulphate		464		464
12	Check		437		437

foliage was badly affected with scab and after midsummer the leaves were cracked, with many dead areas, and were harsh and dry and not nearly so attractive to the mites as the clean, sprayed foliage on the treated plots. No matter what other factors may be present, scabby foliage does not afford the same favorable conditions for red mite as sprayed, healthy foliage, and, if not the only factor, is certainly an important reason why untreated orchards are seldom severely attacked. By October 15, all the foliage had dropped from the check trees and a much larger percentage of the winter eggs must have been laid by the third and fourth generation females here in comparison with those on the sprayed plots.

It will be observed from the table that the mite counts on November 3 were higher than on October 19. On November 3, at least 90% of the foliage had turned yellow, and the red mite always deserts the yellow and migrates to the green, healthy leaves. At the former date the only green foliage on the treated plots was one to three leaves of the terminal growth, all of the remainder having turned yellow, although only a few had fallen. All the adult females had concentrated on these green leaves. When mite counts were made on November 3, only these green leaves were selected. This resulted in a higher mite count than would have occurred had the mites been distributed over the trees.

In making counts of winter eggs on these plots, difficulty was experienced in devising a method that would be accurate. However, terminal twigs were selected, eliminating the current year's growth, ten representative twigs

being taken from each plot. These were measured six inches back of the current year's growth and the winter eggs counted on the measured portion. This gave sixty inches of limb from each plot. Comparing one plot with another, this method gave fairly accurate results.

The results as shown in Table I indicate what can be expected from the use of these standard sprays, as far as red mite is concerned. Observations made at various times in other infested orchards helped further to corroborate these results. Of the sulphur sprays the straight lime-sulphur was the most effective in holding the red mite in check. This was followed by aluminium sulphate mixture, with iron sulphate mixture the least effective. Bordeaux without doubt promoted the increase of red mite in some manner, over either sprays or check. The check had, next to the lime-sulphur plot, the smallest number of winter eggs. With such severe scab infection on the check trees as occurred in 1932, a low mite count was almost inevitable. Although the various predators were more in evidence throughout the season on the check than on the treated plots, it is quite conceivable that a greater mite population would have occurred had there been only a slight or moderate infection of leaf scab.

The following are the individual predators that have been observed attacking the European red mite, with their relation to the economic status of this pest in Nova Scotia apple orchards.

Seiulus pomi Parrott

This mite is probably the most important of all the predators attacking the European red mite in Nova Scotia orchards, not that it consumed more mites during the many periods under observation in comparison with other predators, but on account of its being active for the entire season from early spring before red mite eggs hatch, until well after all winter eggs have been deposited. This predator was also found more generally disseminated than others. The predaceous habits of *Seiulus pomi* Parrott are emphasized by Newcomer and Yothers (1), who observed that the European red mite eggs, and occasionally the immature forms, were consumed, but further stated that adults were not attacked. The writer has observed the adults and more mature nymphal stages of *Seiulus pomi* Parrott attacking all stages of *Paratetranychus pilosus* C. and F. Eggs probably represent the larger proportion of the total consumed, but the immature forms, more particularly during the quiescent periods, were readily attacked. It was also a common occurrence to observe adults, both male and female, being seized and the contents of the body emptied.

The adults of *Seiulus pomi* Parrott hibernate during the winter on the twigs, concealed in such places as under rough bark, in old bud-moth hibernacula and even under deserted oyster shell scales. The mites are active at rather cool temperatures and consume the European red mite winter eggs on the warmest days until late in the autumn. Feeding is again resumed in the spring, a considerable time before the mite eggs hatch. In fact, it may be

that a limited amount of feeding upon the eggs may occur during the warmest days in the winter. The hibernating mites on cold winter days become practically as active as during the summer if brought to the laboratory and placed for a few minutes in a warm room. At such times, however, actual feeding on the eggs of the European red mite was not observed.

Table II, giving counts of winter eggs of red mite, will suggest just what reduction of living eggs takes place between the autumn and the following spring. The method of selecting branches for winter egg counts was similar to that already described. The autumn and spring counts were made from twigs taken from the same trees in the various plots and in as like manner as possible. The sucked out eggs are white and can readily be distinguished from normal healthy ones. There was some chance for error in making the winter egg counts, but there is a wide difference in the number of normal eggs in the autumn and in the spring.

TABLE II

NUMBERS OF EGGS OF EUROPEAN RED MITE ON APPLE TWIGS IN AUTUMN OF 1931 AND SPRING OF 1932, BERWICK, N.S.

Summer treatment	Date of counts	Number of normal eggs	Percentage reduction
Bordeaux spray	Nov. 11/31	3035	
	May 12/32	599	80.3
Lime-sulphur–spray	Nov. 11/31	187	
	May 12/32	87	53.5
Lime-sulphur–iron sulphate	Nov. 11/31	658	
	May 12/32	344	47.7
Lime-sulphur–aluminium sulphate	Nov. 11/31	464	
	May 12/32	189	59.1
Check	Nov. 11/31	437	
	May 12/32	86	80.2

Many factors in addition to predators probably contribute toward this reduction of winter eggs, *e.g.*, fungus diseases, heavy winds, frosts, sleet storms, desiccation, extreme low temperatures with varying climatic conditions, and possibly small birds. From observations made during the past two years it would appear that predators are the most important, and that *Seiulus pomi* Parrott is one of the most efficient of these in the Annapolis valley orchards.

An attempt was made to rear *Seiulus pomi* Parrott on potted apple seedlings in order to obtain at least a general life history and further details of the habits of this predaceous mite. It did not, however, adapt itself to such artificial conditions and this method out of necessity was abandoned. A low growing apple tree was partially caged with cotton and individual limbs used for life history observations. Apparently there are three generations of *Seiulus pomi* Parrott under the climatic conditions of Nova Scotia.

In 1932, the eggs of the first generation were deposited by the hibernating females beginning the last week of May. Eggs were most numerous during the first week of June. The last or third generation eggs reached maximum numbers during the last days of August. The last egg was observed on September 12. The second or intervening generation is approximately midway between the first and third generations. The incubation period varied from 7 to 10 days. It was not definitely determined just how many eggs each female deposited, but the general range was from 6 to 12, in any event not a large number.

The eggs are pearly white in color, ovate and very small, being only .062 mm. at the greatest, and .052 mm. across the smallest diameter. They are deposited on the undersides of the leaves near the midrib or larger veins. Apparently they are not attached to the leaf, but held in place by the leaf hairs.

As a rule the adults of *Seiulus pomi* Parrott are pale without characteristic markings. An occasional individual with pale brownish markings on the dorsum, and a few with the dorsum a pale reddish or wine color were observed. The latter coloration was apparently due to the mites gorging themselves on European red mite eggs, the color showing through the integument. The adults are more frequently found near the midrib and usually on the under surface of the leaf. They give the impression of sluggishness, but when disturbed they move rapidly over the leaf. The main difference in appearance between the adult and young mites is in size, although the latter are usually paler, being practically white in the younger stages.

It was possible to rear *Seiulus pomi* on foliage in the absence of the European red mite, the leaf juices being their only food. The species is, therefore, not entirely carnivorous. A special effort was made to obtain data relative to the rate of consumption of red mites by this predator. The results are given in Table III:—

TABLE III

RATE OF CONSUMPTION OF EUROPEAN RED MITE, IMMATURE FORMS, BY THE OVERWINTERING *Seiulus pomi* PARROTT ADULTS

Date	Number of *S. pomi* on each spur	Number of *P. pilosus* nymphs on each spur	Number of nymphs after ten days	Reduction, %	Average number of immature forms consumed per day
May 20	2	90	25		
23	1	16	2		
23	3	32	7		
26	2	45	7		
26	3	25	1		
27	2	115	40		
27	1	39	12		
28	1	24	10		
30	2	30	9		
30	2	85	25		
		501	148	72.45	1.91
May 25	Check	74	73		
25	Check	77	75		
30	Check	22	22		

In this experiment the average consumption per day by each adult *Seiulus pomi* Parrott was 1.91 European red mites. This was a reduction over the ten day period of 72.45%. At this rate about 26 immature forms of the first brood would be consumed by each predator. This is considering a period of only 14 days which was the average duration of the immature stage of the European red mite at this season. Actually the period would extend to 20–25 days as all do not mature at the same time and the number of mites consumed by each predator would be about 40 rather than 26. Of course, the percentage of mites consumed in a given feeding experiment would vary according to the number present. In the event of the European red mites being greatly increased in proportion to the predaceous mites, the percentage consumed would be decreased accordingly. It would seem, however, that the average rate of consumption per day should remain at least fairly constant.

Seiulus pomi Parrott was found in all infested orchards examined during 1932. In some the number was not great while in others they were relatively numerous. Table IV shows the results of leaf counts made in various orchards to obtain the number of *Seiulus pomi* Parrott per leaf.

TABLE IV

AVERAGE NUMBER OF THE PREDACEOUS MITE, *Seiulus pomi* PARROTT, PER LEAF IN ORCHARDS IN VARIOUS LOCALITIES IN THE ANNAPOLIS VALLEY, 1932

Date of count	Orchard number	Number of leaves counted	Number of *S. pomi*	Average per leaf
Aug. 30	1	100	445	4.45
Sept. 8	2	100	102	1.02
8	3	100	26	0.26
14	4	100	210	2.10
14	5	100	210	2.10
30	6	100	67	0.67
30	7	100	153	1.53
30	8	100	373	3.73
30	9	100	185	1.85

Further feeding experiments were conducted by placing a certain number of adult predaceous mites and newly emerged female European red mites on individual apple spurs. From this experiment there was obtained a fairly accurate indication of the number of European red mite eggs present on the foliage after a period of 10 days, with an association of predator and host *versus* an equal number of female European red mites but no predator. The results of this feeding are presented in Table V.

In most orchards there appeared to be a direct relation between the numbers of predaceous mites and of the European red mite. This apparently occurred except where the orchard practice has been such as to affect directly or to prevent this approximate balance between host and predator. It is certain that some of the sprays ordinarily used in the Annapolis valley, not only control injurious pests but at the same time kill many beneficial predators. As a result the natural balance is thrown out of adjustment in favor of the pest.

TABLE V

AVERAGE NUMBER OF RED MITE EGGS ON FRUIT SPURS IN THE PRESENCE AND ABSENCE OF PREDACEOUS MITES AFTER A 10 DAY PERIOD, ANNAPOLIS ROYAL, N.S., 1932

Date	Number of *S. pomi* adults on each spur	Number of adult females of *P. pilosus* on each spur	Number of eggs of *P. pilosus* after ten days	Average number of eggs of *P. pilosus*	Per cent reduction eggs over check
June 8	2	7	132		
8	2	7	148		
8	2	7	124		
8	2	7	39		
8	2	7	146		
				16.6	
8	Check	7	267		
8	Check	7	256		
8	Check	7	249		
				36.7	54.7
13	7	44	972	20.1	
13	Check	44	1563	35.5	37.7

A concrete example may be cited. Dormant oil spray not only destroys most of the winter eggs of the European red mite, but also kills the larger proportion of the hibernating adults of *Seiulus pomi* Parrott, thus largely removing probably the most important natural controlling factor. It follows, therefore, that when dormant oil sprays have once been resorted to in controlling European red mite, they must, as a rule, be applied about every two years as the mites increase again very rapidly, the natural control apparently being so reduced as to be ineffective.

A test of the various standard sprays was made to determine to some extent, the effect of such sprays on *Seiulus pomi* Parrott. This was done by transferring the mites to cells one inch in diameter clipped to a leaf. Results are presented in Table VI.

TABLE VI

RESULTS OF VARIOUS SPRAYS ON THE PREDACEOUS MITE, *Seiulus pomi* PARROTT, ANNAPOLIS ROYAL, N.S., 1932

Date	Number of adults	Spray	Number dead in 5 hr.	Number dead in 24 hr.	Number dead in 48 hr.	Remarks
Oct. 1	30	3% oil in 3 : 10 : 100 Bordeaux	20	30		
6	30	Bordeaux 3 : 10 : 100 plus 1 lb. arsenate lime	30			
6	39	Lime-sulphur 1 : 40, arsenate lead, 1 : 40	0	2	10	
6	15	Check	0	0	0	
7	32	Nicotine 1 : 800	0	0	0	
8	30	Standard lime-sulphur, iron sulphate mixture	25	26	26	Live mites feeble after 24 hr.
8	26	Standard lime-sulphur, aluminium sulphate mixture	15	26		
8	20	3% Sunoco oil	10	15	20	
Dec. 29	25	3% Sunoco oil	10	25		

In some respects this test must be considered as severe, for not only were the mites well covered with spray but they were actually immersed for some time in the solution as it more or less filled the cell. Nicotine evidently has no detrimental effect upon this predator, and lime-sulphur was the only spray that did not completely destroy the mites eventually. Ordinarily actual spraying would not so thoroughly cover such a large percentage of these predators as in the above experiment, therefore it is doubtful if more than a small proportion of the predators would be killed in the application of lime-sulphur. Further, when oils are applied in the dormant season many of the predators would be more or less concealed as already mentioned and 100% contact could not be expected.

We would not like to consider these results conclusive as the test was entirely too limited. However, the results are suggestive and indicate the probable effect upon this predator, of sprays ordinarily used in Annapolis valley orchards.

Diaphnidia pellucida Uhl.

This mirid, identified in 1930, was one of the first predators to be observed attacking the European red mite in Nova Scotia orchards.

In both the nymphal and adult stages it was very active and fed upon all stages of the mites. It was interesting to observe the movements of these insects when searching for their host. They did not search promiscuously but were very methodical, proceeding slowly and deliberately over the leaf surface using their beaks to search for prey. The beak was moved rapidly from side to side between the front pair of legs, completely covering a portion of leaf about $\frac{1}{16}$-in. in width. By proceeding slowly in this manner in an irregular course over the leaf they located the various stages of the mites. The beak is an extremely sensitive organ by which all food is located. While actually searching for food as described their legs or other parts of the body frequently come in contact with a mite, but this in no way appeared to direct them toward their food. When capturing the immature forms, or an adult female mite, they become quite excited, moving quickly and skilfully. The host is held firmly with the front legs and the beak quickly inserted, withdrawing the contents of an egg is about one minute. It takes only slightly longer to empty the body of an adult mite. The beak is usually inserted only once in an egg but frequently is withdrawn from an adult and again inserted in another part of the body until there remains only the outer skin. The empty eggs at once become transparent but do not collapse, remaining on the leaf for some time in their original form. Owing to the greater activity of the male in comparison with the female mites, the former were only occasionally captured.

It was found so difficult to rear this capsid in confinement that this part of the investigation was practically abandoned. The nymphs soon died in the cages and the adults also lived but a short time and produced no eggs. The following life history notes were from necessity obtained entirely from field observations.

The winter is evidently passed in the egg stage, as the first appearance of the insect in 1932 was on June 15, when the newly hatched nymphs were observed. The duration of the nymphal period was about three weeks. It should be mentioned in this connection that stray nymphs and adults were seen on many occasions during August, and the latest observed was on September 14. No detailed comparisons were made between these late appearing individuals and those observed when the numbers were at a maximum during June and July, but a superficial examination revealed no difference. However, there is the possibility that another closely related species may be involved.

The predaceous habits of this insect were consistent, and in the aggregate large numbers of mites were consumed, the measure of control depending, of course, upon the numbers present. The period when most mites were consumed was from about June 15 to July 15 or possibly a little later. This predator was found to be well disseminated in most infested orchards and often sufficiently numerous to effect a great reduction of the pest. In other instances results were not so apparent owing to lack of numbers. Of the many predators attacking the European red mite in Nova Scotia, *Diaphnidia pellucida* Uhl. is one of the most important.

Table VII will convey some idea of the rate at which red mites are consumed by *Diaphnidia pellucida* Uhl.:—

TABLE VII

NUMBERS OF EUROPEAN RED MITES CONSUMED BY *Diaphnidia pellucida* UHL. IN NYMPHAL AND ADULT STAGES IN CONSECUTIVE HALF-HOUR PERIODS, ANNAPOLIS ROYAL, N.S., 1932

Date	Period of observation	Predator	Numbers consumed				
			Eggs	Immature forms	Adult female	Adult male	Total
July 15	1st half hr.	Nymph	3	3	2	1	9
	2nd half hr.	Nymph	4	3	1	2	10
	3rd half hr.	Nymph	2	4	0	0	6
	4th half hr.	Nymph	3	2	2	0	7
			Total consumed in 2 hr.				32
	1st half hr.	Adult	5	5	3	1	14
	2nd half hr.	Adult	6	3	3	0	12
	3rd half hr.	Adult	6	4	4	1	15
	4th half hr.	Adult	5	3	2	0	10
			Total consumed in 2 hr.				51
Aug. 8	1st half hr.	Nymph	1	9	4	0	14
	2nd half hr.	Nymph	1	1	2	1	5
	3rd half hr.	Nymph	1	2	0	1	4
			Total consumed in 1½ hr.				23
	1st half hr.	Nymph	2	14	2	1	19
	2nd half hr.	Nymph	5	9	4	0	18
			Total consumed in 1 hr.				37

This predator, like *Seiulus pomi* Parrott, may be greatly reduced in numbers through the ordinary spraying operations. The nymphs are quickly killed with nicotine sprays. If, therefore, nicotine is included in the later sprays, large numbers of this predator are killed. It was also found that nicotine had little effect upon the adults. This was largely due to their habit of taking wing when the first spray hit the tree and thus avoiding contact. Even those receiving a mist were not killed, owing to the coating of fine hairs which prevents the wetting of the body. It was only with severe drenching that any mortality was observed, and this seemed to occur only on rare occasions.

Diaphnidia capitata Van D.

This species closely resembles *Diaphnidia pellucida* Uhl. in many respects. The nymphs of the two species are difficult to separate in the field. The adults, however, are easily distinguished as *capitata* is slightly smaller, and has a black head whereas in *Diaphnidia pellucida* Uhl. the head is pale greenish. Not a great deal is known of the life history of *Diaphnidia capitata* Van D., but as near as could be determined, the seasonal appearance of these two species was much the same. The nymphs of *Diaphnidia capitata* Van D. were first observed in 1932, on June 19, and the adults in the latter half of July. Both the nymphs and adults fed on all stages of the European red mite, the rate of consumption being much the same as with *Diaphnidia pellucida* Uhl. In Table VIII is shown the rate at which European red mites were consumed by the nymphs of *Diaphnidia capitata* Van D.

TABLE VIII

NUMBERS OF EUROPEAN RED MITES CONSUMED BY *Diaphnidia capitata* VAN D., IN CONSECUTIVE HALF-HOUR PERIODS, ANNAPOLIS ROYAL, N.S., 1932

Date	Predator	Time	Numbers consumed				
			Eggs	Immature forms	Adult female	Adult male	Total
July 15	1. Fifth instar	1st half hr.	1	7	1	0	9
		2nd half hr.	0	11	5	0	16
		3rd half hr.	2	5	3	0	10
		4th half hr.	4	4	4	0	12
							47

Diaphnidia capitata Van D. is of much less importance in the Annapolis valley than *Diaphnidia pellucida* Uhl. as it is less numerous. Although it was observed frequently at widely scattered points, in no instance were its numbers sufficient to cause any appreciable reduction in the European red mite population. Like *Diaphnidia pellucida* Uhl. the nymphs were quickly killed with nicotine spray.

Hyaliodes vitripennis Say

One of the common predators of the European red mite is the mirid, *Hyaliodes vitripennis* Say. This predator, in both nymphal and adult forms, was observed feeding upon all stages of the mite. The habits of this insect

and its method of capturing and feeding upon mites were similar to those of the capsids which have been previously mentioned. It usually takes about one minute to empty an egg, and from one to two minutes to remove the body fluid of an adult mite. As a rule only the collapsed skin of the mite remains, but occasionally the body content is only partially removed, always resulting, however, in death to the mite. On one occasion a nearly mature nymph of *Hyaliodes vitripennis* Say attempted repeatedly to puncture with its beak an adult of the predaceous mite *Seiulus pomi* Parrott. This it was unable to do, as the dorsum of the mite proved too hard to be penetrated.

When the appetite of *Hyaliodes vitripennis* Say is temporarily satisfied, it spends considerable time at rest, on the leaf, cleaning or stroking its antennae and beak. This is done by seizing these organs with the front pair of legs and drawing them between for their entire length. The nymphs when moving over the leaf usually carry the caudal portion of the abdomen in a partially raised position, frequently nearly vertical.

In Table IX is shown the rate of consumption of all forms of the European red mite by the nymphs of *Hyaliodes vitripennis* Say:—

TABLE IX

NUMBERS OF EUROPEAN RED MITES CONSUMED BY *Hyaliodes vitripennis* SAY, IN CONSECUTIVE HALF-HOUR PERIODS, ANNAPOLIS ROYAL, N.S., 1932

Date	Predator	Time of observation	Numbers of mites consumed				
			Egg	Immature forms	Adult female	Adult male	Total
July 8	2nd instar	1st half hr.	3	7	0	2	12
		2nd half hr.	1	8	0	0	9
		3rd half hr.	0	3	0	0	3
							24
July 8	2nd instar	1st half hr.	0	8	0	1	9
		2nd half hr.	1	11	0	0	12
		3rd half hr.	0	3	0	1	4
							25
July 9	2nd instar	1st half hr.	1	10	0	3	14
		2nd half hr.	0	9	1	1	11
		3rd half hr.	0	2	2	0	4
							29
July 9	2nd instar	1st half hr.	0	10	0	3	13
		2nd half hr.	0	9	1	1	11
		3rd half hr.	1	1	0	0	2
							26
July 15	4th instar	1st half hr.	3	4	13	0	20
		2nd half hr.	3	0	5	0	8
		3rd half hr.	11	3	6	1	21
		4th half hr.	12	3	8	1	24
							73
July 15	3rd instar	1st half hr.	1	1	6	0	8
		2nd half hr.	3	0	6	1	10
		3rd half hr.	12	0	4	0	16
		4th half hr.	3	2	2	0	7
							41

The newly hatched nymphs of *Hyaliodes vitripennis* Say began to appear in orchards in 1932 during the last days of June. The nymphal period lasts for about four weeks. The first adult was recorded on July 25 and they were numerous during the first days of August. After August 7 they began to decrease rapidly in numbers. Although the eggs were not found, the fact that the nymphs appear upon the trees in the spring indicates that they must be deposited during the latter part of the summer, somewhere about the trees. There is only one brood in a season.

This bug is one of the most important enemies of the European red mite in the Annapolis valley. It is larger than the two capsids already discussed and consumes relatively more mites. Apparently the nymphs are somewhat more persistent in their predaceous habits than the adults. The latter are, however, predaceous feeders to a very considerable extent. It is unfortunate that the feeding period of this group of predators does not extend over a greater portion of the season. The period of activity of *Hyaliodes vitripennis* Say is from the last week of June to about the end of the first week of August, a period of about five to six weeks. During this part of the summer of 1932 many European red mite infestations were effectually held in check by this predator.

Nicotine was very toxic to the nymphs of this species also. The adults, owing to their activity escaped to a very large extent when the spray was applied. The application of sprays including nicotine after June 25 would destroy most of the nymphs of this predator.

Stethorus punctum Leconte

This is a small species of coccinellid beetle closely related to the western form *Stethorus picipes* Casey, which is an important predator of the European red mite in the Pacific Northwest.

On July 21, 1932, the larvae of *Stethorus punctum* Leconte were observed in a mite-infested orchard near Bridgetown, N.S. Observations were made for a few minutes only and no mite-feeding habits noticed. A week later its predaceous habits were observed. The larvae of the beetle crawl slowly over the leaf, devouring the mites as they come into contact with them. Eggs and the young mites were the forms chiefly consumed. As the larvae move comparatively slowly adult mites often escaped. However, adults occasionally were seized and devoured.

Table X shows the numbers of mites consumed by the beetles, during consecutive half-hour periods of observations.

Very little is known of the life history of this species of predator. When first observed on July 21 the larvae were more than half grown. Adults were first observed on July 28, and at that date there were about equal numbers of larvae and pupae. The adults are very small; those measured being from 1.3 to 1.5 mm. long. They are black with brownish legs, and the wing covers are clothed with short hairs. After the adults emerged they did not

TABLE X

NUMBERS OF EUROPEAN RED MITES CONSUMED BY *Stethorus punctum* LECONTE, IN CONSECUTIVE HALF-HOUR PERIODS

Date	*Stethorus punctum*	Time	Numbers of mites consumed				
			Eggs	Immature forms	Adults		Total
					Females	Males	
July 29	Larva	1st half hr.	0	8	0	0	8
		2nd half hr.	1	4	0	0	5
		3rd half hr.	3	1	0	0	4
		4th half hr.	0	5	0	0	5
		5th half hr.	2	5	0	0	7
		6th half hr.	1	1	0	0	2
			Total consumed in 3 hr.				31
July 29	Larva	1st half hr.	0	8	0	0	8
		2nd half hr.	2	4	0	0	6
		3rd half hr.	1	0	0	0	1
		4th half hr.	1	3	0	0	4
		5th half hr.	0	0	0	0	0
		6th half hr.	1	3	1	0	5
			Total consumed in 3 hr.				24
July 30	Adult	1st half hr.	5	8	1	1	15
		2nd half hr.	3	0	0	0	3
		3rd half hr.	1	1	1	0	3
		4th half hr.	3	4	1	0	8
			Total consumed in 2 hr.				29
Aug. 3	Adult	1st half hr.	1	10	0	0	11
		2nd half hr.	1	5	0	0	6
		3rd half hr.	1	1	0	0	2
		4th half hr.	0	5	1	0	6
			Total consumed in 2 hr.				25
Aug. 3	Adult	1st half hr.	0	5	1	0	6
		2nd half hr.	0	4	1	0	5
		3rd half hr.	0	1	0	0	1
			Total consumed in 1½ hr.				12
Aug. 4	Adult	1st half hr.	0	3	0	0	3
		2nd half hr.	0	3	0	0	3
		3rd half hr.	Apparently slept through this period				
		4th half hr.	0	3	1	0	4
			Total consumed in 2 hr.				10
Aug. 4	Adult	1st half hr.	2	8	1	0	11
		2nd half hr.	2	3	1	0	6
		3rd half hr.	0	3	0	0	3
			Total consumed in 1½ hr.				20

remain long upon the trees. In all probability there is only one generation in Nova Scotia, and the most reasonable assumption is that the adults hibernate in proximity to the orchard.

The larvae spend most of their time in crawling over the leaf surface. Apparently this activity was for the purpose of obtaining food. Whenever contact is made with a host it is seized and the contents sucked out. The larvae usually spend from three to five minutes emptying the contents of an egg or the body of an immature form, and from five to eight minutes in consuming an adult.

The adult beetles crawl quickly over the leaf. Apparently there is no instinct directing them toward their host, and it is simply by chance that they come upon the mites in their irregular course. Even with only a few mites on a leaf, it is remarkable how adept these creatures are at finding and devouring one mite after another. As a rule they consume only the interior of the mites, but sometimes they devour the whole body. The contents of an egg or young mite is usually devoured in about one-half to one minute, and an adult mite in from one to two minutes. When the appetite of the beetle apparently has been satisfied it may remain at rest on the leaves for periods of one-half to one hour. When thus at rest it is entirely in repose as if asleep. If disturbed it usually only crawls to another position and assumes the same attitude, as if resenting the disturbance.

It is apparent that this predator is one capable of destroying large numbers of mites. In a number of orchards, during the summer of 1932, from one to three larvae were present on many of the leaves. In these orchards very material reductions of the pest occurred in a comparatively short time. Should *Stethorus punctum* Leconte become somewhat more numerous and more widespread, it would probably rank next to *Seiulus pomi* Parrott in value as a predator. Notwithstanding lack of lumbers, it must still be considered as one of the more important predators attacking European red mites in the Annapolis valley.

No apparent ill effects to the adults or larvae were noted when spraying with nicotine solution. The minute hairs which clothe the body seem to prevent the insect from becoming wet with the spray.

Plagiognathus obscurus Uhl.

Late in July and early in August, 1932, the adults of *Plagiognathus obscurus* Uhl. were observed in a number of orchards, particularly at Lakeville, N.S. There were no nymphs present at this time and, owing to the fact that none were observed on any previous occasion, it seems that the adults may have flown in from some other host plant.

The adults particularly frequented the terminal growth where green apple aphids were present, from 10 to 12 being present on some terminals. In a few instances they were observed to pierce an aphid and wholly or partially suck out the contents of the body. On different occasions they were also observed feeding on all stages of the European red mite in a like manner.

Mr. G. S. Walley, Entomological Branch, Ottawa, who identified the species, states that members of the genus *Plagiognathus* are phytophagous and that the above species is usually taken in flower clusters, commonly

on Compositae. This would lead further to the belief that *Plagiognathus obscurus* Uhl. does not breed on apple and that possibly this predaceous habit is somewhat unusual.

The adults were very shy and at the least disturbance dropped from the tree, but took wing before reaching the ground, and would alight upon another branch. On account of their elusive habit it was difficult to observe their feeding for any extended period. However, an adult which was observed for a ten-minute period consumed two eggs, three immature forms, and one male adult of the European red mite. It would appear from what little is known of *Plagiognathus obscurus* Uhl. that possibly its predaceous habits may not be very consistent. However, in the few orchards where they were most numerous large numbers of mites were consumed.

Camptobrochis nebulosus Uhler

A few nymphs of this species were observed feeding on European red mites in an orchard near Bridgetown, N.S. The nymphs were reared and the adults identified by Mr. G. S. Walley. This species is a member of the subfamily Deraecorinae to which belong a number of species of predatory miridae. The nymphs were first observed on July 29 and at this date were all in the last instar. Adults began to appear on August 2, but nothing further is known of the life history of this insect. The nymphs were so near maturity that there was little opportunity to observe their predaceous habits. However, on a few occasions they were observed for ten minute periods. During these short observations each nymph consumed on the average one egg, one adult and four immature forms. This predator was not found in sufficient numbers in any orchard during 1932 to have any appreciable effect upon the mites. The writer recalls observing the nymphs of this species at Wolfville, N.S., in 1924 and 1925, and as nearly as can be remembered, they were considerably more numerous at that time than during the summer of 1932.

The predators which have been discussed above are apparently the most important that attack the European red mite in Nova Scotia orchards. However, several other predators, the value of which it was difficult to estimate, were observed feeding on mites from time to time. Had these been observed in greater numbers more data could have been obtained relative to their predaceous habits. The following are the minor predators:—

Anystis agilis Banks

This is one of the larger mites and is bright red in color. It runs rapidly over the foliage in a haphazard manner, evidently in search of food. Banks mentions that it feeds upon aphids, small caterpillars and young larvae of the currant sawfly. The writer has noticed it feeding on the European red mite on several occasions. Apparently whenever *Anystis agilis* Banks in its rapid movement over the foliage chanced upon the various forms of *Paratetranychus pilosus* C. and F., it seized and devoured them. In the vicinity of Annapolis Royal, *Anystis agilis* Banks was common. In fact it was thought

sufficiently numerous to have an appreciable effect in reducing the European red mite. It was in late June and early July when most were observed. In the main fruit belt where spraying was general this predator was observed only on rare occasions.

Thrips

On several occasions an unidentified species of thrips was observed to attack young and adult mites. The body contents of the mites were sucked out in some manner. These thrips were observed during late August and early September and all at that time were in the immature stages. It was evident that this species of thrips is a more or less general predator for it was observed to seize and suck the body fluids of a leaf hopper nymph as well as the mite *Seiulus pomi* Parrott. During 1931 this thrips was seen frequently, and in a few instances was fairly abundant, but in 1932 very few individuals were observed. It is only in the event of this predator becoming sufficiently numerous that it possibly could be rated as an important natural control factor in relation to the European red mite.

Adults of this thrips have not been observed. A few immature forms were forwarded to the Entomological Branch, Ottawa, for identification, but these proved insufficient. It was stated, however, that they probably belonged to the genus *Haplothrips*. Viewed from above, the color marking of the fully grown larva of this thrips was as follows:—Prothorax red on lateral margins, darker on dorsum; mesothorax yellowish; metathorax red; Abdominal Segments 1 and 2 yellowish with some fine dark pepper markings; 3 and 4 red; 5 and 6 yellowish; 7 and 8 red with a few dark markings; taking Segments 9 and 10 together, the first third black, followed by a narrow transverse band of red with the cauda black.

Syrphid Larvae

On a few occasions syrphid fly larvae were observed to seize a quiescent European red mite and consume the larger portion of the body. It is not likely that these larvae could capture adults or active young mites, and it is not known whether they would favor mite eggs. Under the most favorable circumstances it is not likely that this group of predators will become important enemies of the European red mite. It is necessary that the larvae have near at hand an abundance of food such as aphid clusters, among which they are frequently found. The feeding observed upon mites was probably more or less a chance occurrence.

Campylomma verbasci Meyer

This is a small capsid bug, the adults of which were observed to be very numerous in a few orchards during 1931. The adults of this predator were observed attacking only the adult mites, but eggs may also be consumed. These bugs were the most numerous on July 9, and only a few days later they

had largely disappeared. This insect has two generations in Nova Scotia. The first brood adults migrate to the potato plant where they feed for some time before returning to the apple.

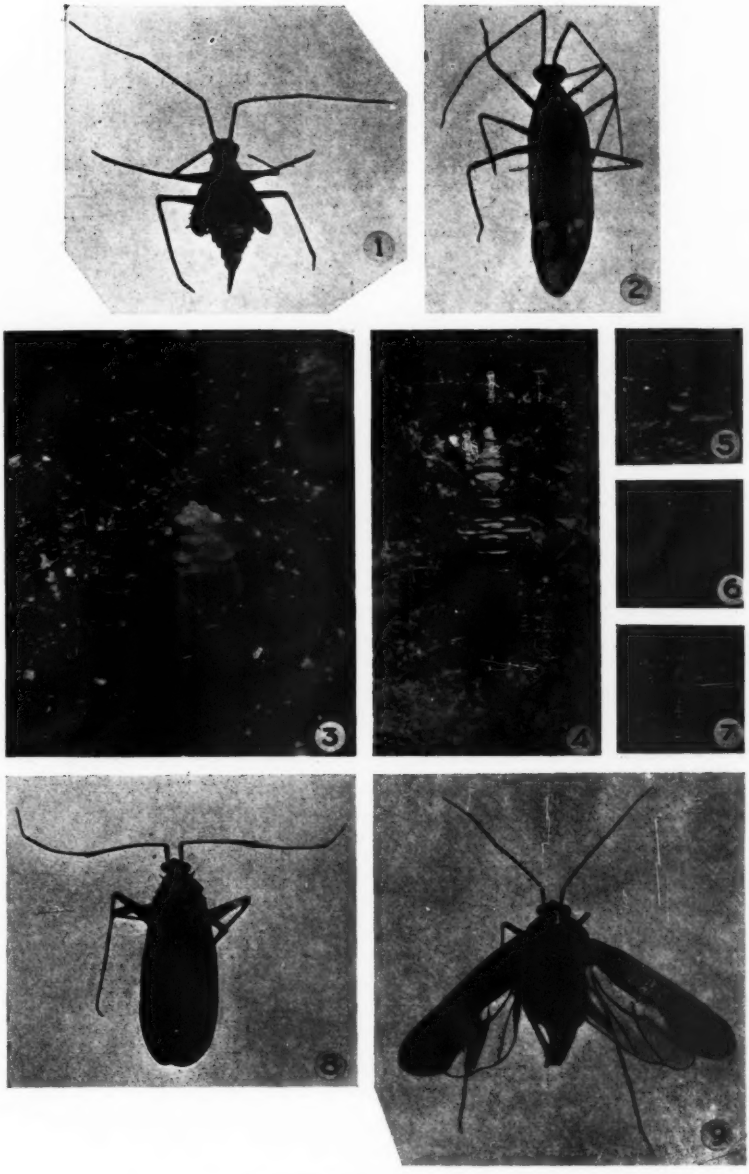
The adult bugs do not search in any systematic way for mites as other capsids do, but seize them as they chance upon them in their erratic movement over the foliage. They frequent the terminal growth of the apple, especially trees infested with aphids. They were not observed feeding upon aphids but it seems likely that this occurs. One adult stung the back of the writer's hand sharply.

While these bugs were present large numbers of mites were consumed. This was very evident in one orchard. However, although large reductions of mites may occur on some occasions, this is not considered a predator of any great consequence. Its appearance in numbers is too infrequent and its stay is apparently very short. During 1932 this insect was observed only on a few occasions and then in small numbers.

Reference

1. NEWCOMER, E. J. and YOTHERS, M. A. Biology of the European red mite in the Pacific Northwest. U.S. Dept. Agr. Tech. Bull. 89. 1929.

PLATE I



1, Last stage nymph of Hyaliodes vitripennis Say; 2, H. vitripennis Say, adult; 3, Last stage nymph of Diaphnidia pellucida Uhl.; 4, Unknown predator on European red mite, probably Nabid; 5, Egg of Seiulus pomi Parrott; 6, Egg of Campylomma verbasci Mey.; 7, Seiulus pomi Parrott, adult; 8, Diaphnidia pellucida Uhl., adult; 9, Plagiognathus obscurus Uhl., adult.